THE HÆMOLYTIC ANÆMIAS

Congenital and Acquired

PART I—THE CONGENITAL ANÆMIAS

By

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PREFACE TO THE SECOND EDITION

In the preparation of a new edition of this book I have been confronted with some difficult problems Our knowledge of the blood and its diseases continues to advance at a tremendous rate and the number of new papers which have been published in the last five years dealing with the hæmolytic anæmias and allied fields must run into thousands In particular the pace of advance in knowledge of the abnormal hæmoglobins and the syndromes to which they give rise has been breathtaking The incorporation of even the more important parts of this new information into the framework of the old has been a formidable task and has led to an almost complete re writing of the book The size of the task and the ever diminishing time that I have been able to devote to it explains why the book is appearing in two parts To complete the whole would have meant postponement of publication for at least another year perhaps longer

The present volume (Part I) includes the introductory Chapter and the five chapters devoted to the congenital hæmolytic anæmias and the hæmoglobinopathies Part II when completed will deal with the acquired and secondary hæmolytic anæmias the drug induced hæmolytic anæmias paroxysmal nocturnal hæmoglobinuria and hæmolytic disease of the newborn

I have kept to the aim I had in writing the first edition that is to say I have attempted to provide an up to date and reasonably complete reference book useful to Physicians and Pathologists interested in blood diseases To cover adequately and completely the literature of the whole world would be an almost superhuman task and I certainly have not succeeded in doing this However I hope that I have not overlooked too many papers of major importance and that I have given due credit to the pioneer workers in the various fields touched upon I have omitted for reasons of space the few case reports which were to be found in the corresponding chapters of the first edition of this book

Facts or opinions which are generally accepted and which are not controversial have usually been set out without any specific references except where reference is made to the original author(s) or where 'key references have been given The authors of relatively new or less well substantiated information have on the other hand generally been named In the absence of any reference

a statement or opinion may be assumed to be in line with my own view of the subject

As before I have not hesitated to include observations made on patients I have studied personally and I have been greatly helped by my colleagues Drs J C White D L Mollin and S M Lewis and other past and present members of the medical and technical staff and students of the Postgraduate Medical School who have allowed me to quote observations they themselves have made I am also exceedingly grateful to many friends and colleagues in other hospitals who have been so good as to refer patients to me

I should like to record my gratitude to Dr H Lehmann for allowing me to reproduce ten diagrams in Chapters 5 and 6 Some of the diagrams have appeared in a slightly different form in *The Abnormal Hæmoglobins A Symposium* recently published by Blackwell Scientific Publications I am grateful for the publisher s permission to reproduce them and also for allowing me to include the three illustrations which appear as Figs 17 89 and 90 which were originally published in the *British Journal of Hæmatology* I should also like to thank Dr P L Mollison and Blackwell Scientific Publications for permission to reproduce two figures from *Blood Transfusion in Clinical Medicine* 2nd edition 1956 Mrs P A Benyon and Mr F Saunders have finished the illustrations for me and Mr W H Brackenbury has taken the photomicrographs which are new to this edition I am greatly indebted to them and also to Dr S M Lewis for his valued help in proof reading As always I have greatly appreciated the co operation and understanding of the publishers in the preparation of this edition

J V DACIE

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CHAPTER 1

GENERAL FEATURES OF INCREASED HÆMOLYSIS BLOOD PICTURE AND METHODS OF INVESTIGATION OF THE HÆMOLYTIC ANÆMIAS

The essential feature of a hæmolytic anæmia is a reduction of the life span of the patient s erythrocytes As will be shown later this may be due to many different causes This chapter is concerned with the ways in which an increased rate of erythrocyte destruction *in vivo* may be recognized and with the methods which may be used to measure the intensity of increased hæmolysis The clinical effect on the patient and the characteristic changes in the blood picture are also described in general terms and brief reference is made to the structure and metabolism of the erythrocytes and the processes which bring about their destruction in health Finally the significance and importance of certain laboratory tests will be discussed in connection with the diagnosis of hæmolytic anæmia

Certain points of definition need consideration Crosby (1955) pointed out that the term hæmolytic anæmia is often used incorrectly inasmuch as anæmia may be absent although the erythrocytes are being destroyed at an increased rate and that the same is true of the use of the term hæmolytic jaundice for jaundice likewise may be absent He himself uses hæmolytic disease as the generic term and refers to cases without anæmia as suffering from compensated hæmolytic disease Although Crosby is undoubtedly correct in what he says the fact remains that the great majority of patients with hæmolytic disease *are* anæmic it is for this reason that the term hæmolytic anæmia is retained in this book

Another term which needs careful definition is the word hæmolysis It is convenient to refer to increased hæmolysis as indicating simply that erythrocyte destruction is proceeding in the patient at a rate in excess of normal It does not specify the way in which the destruction is brought about and in particular it does not necessarily mean that the cells are being lysed *in vivo* by complement as is usually meant when hæmolysis is used to describe the results of tests *in vitro*

According to Crosby (1952) William Hunter of London was the first to coin and to use the term hæmolytic In his book *Pernicious Anæmia* published in 1901 Hunter referred to the presence of yellow[1] spherical microcytes (Eichhorst s corpuscles) in pernicious anæmia He wrote (p 67) My experiments shew that similar bodies can be produced artificially by action of destructive agents that they mark the anæmia as due to excessive destruction of blood and not to deficient formation *that they denote the anæmia to be hæmolytic not hæmogenic in its* origin [my italics]

Classification The classification of the hæmolytic anæmias presents a number of difficulties and no new or elaborate system will be presented in this work

A clinical classification into acute or chronic cases primary or secondary cases or into cases with or without hæmoglobinuria is of limited usefulness Classifications according to ætiology or pathogenesis are also unsatisfactory because often one or both are unknown or incompletely known

The classification adhered to in this book in which a distinction is drawn between congenital and acquired cases is orthodox separation of the congenital hæmolytic anæmias from the rest is based not only on the concept of a genetic cause but also on a distinct type of pathogenesis the congenital hæmolytic anæmias being the result of various intrinsic abnormalities of the erythrocyte or of its hæmoglobin or are secondary to a definite genetically controlled disorder of hæmopoiesis

The acquired hæmolytic anæmias are an even more mixed collection of anæmias of varied and often unknown causation Some of them may have an as yet undetermined genetic basis as has recently been established in certain drug induced acquired hæmolytic anæmias (see Chapter 15) Most of them nevertheless certainly depend upon pathological processes which affect normal erythrocytes as well as the patient s corpuscles (extrinsic mechanism of hæmolysis) Paroxysmal nocturnal hæmoglobinuria *appears to be unique in that there is no clear evidence for a genetic* basis yet the abnormality is one which seems to be intrinsic to the patient s erythrocytes This fascinating disease is dealt with in the penultimate chapter it is followed by hæmolytic disease of the newborn which is unique in a different way being congenital and acquired (from the mother) yet not hereditary

The separation of the hæmolytic anæmias into two main groups—those due to intrinsic defects of the erythrocyte and those due to mechanisms extrinsic to the cell—has been widely adopted

[1] As viewed unstained

and has been used both by Dausset (1953) and Dameshek (1955) in their classifications

DESTRUCTION OF ERYTHROCYTES IN HEALTH

The Erythrocyte and Its Metabolism

It is reasonable to hope that the progress made in recent years in the understanding of the structure and metabolism of the erythrocyte will help in the elucidation of hæmolytic mechanisms *The erythrocyte although non nucleated is alive after water* its main constituent is hæmoglobin its framework of stroma consists mainly of protein and lipid the latter being concentrated at the surface In addition to water protein and lipids the erythrocyte contains numerous electrolytes and enzymes Many of these constituents are in a constant state of replacement and exchange with the surrounding plasma Hæmoglobin however is an exception The stroma appears to be in a state of flux particularly with respect to the lipids (London and Schwarz 1953 James Lovelock and Webb 1957) Glycolysis continually occurs and is the main energy producing mechanism of the cell Details as to structure and metabolism are given in the recent reviews of Ponder (1954) Prankerd (1955 1956 1959) Crosby (1957a) and Jacobs (1958)

The metabolic activity of young cells (reticulocytes) differs substantially from that of mature cells Reticulocytes retain the ability to synthesize hæmoglobin (London Shemin and Rittenberg 1950) and there is an appreciable aerobic respiration glycolysis too is increased in rate As the reticulocyte matures it diminishes in volume and surface area and apparently loses lipid as it does so (Crosby 1952 Prankerd 1958) Enzymic activity lessens (Allison and Burn 1955)

Life Span of Erythrocytes in Health *As indicated above* there is evidence of lessening in the metabolic activity of the erythrocyte as it matures and it is now generally agreed that it is the exhaustion of enzyme systems which control metabolic activities essential for the integrity of the cell as a whole or of its surface which determines its finite life span (Granick 1949 Ponder 1951 Prankerd 1955 Mollison 1956b) Approximately 1/120th of the total number of circulating erythrocytes of a healthy adult *is destroyed and replaced daily This figure is* based upon estimates of the average life span of the normal erythrocyte which have been derived from several different types of experiment (Callender Powell and Witts 1945 1947 Jope 1946 Shemin and Rittenburg 1947 Mollison 1956a)

It is generally agreed that the erythrocytes of both men and women survive for approximately the same length of time However whereas the curve of elimination in men is normally a straight line it is often slightly curvilinear in women It is doubtful whether this can be accounted for entirely by loss of cells at menstruation and there is a suspicion that a certain amount of random destruction takes place It is highly probable that even in health not every cell survives for exactly the same time in the circulation *i e* there is some scatter around the mean cell life span but the extent of this scatter is not exactly known It is possible too in health that a small proportion of very short lived cells are also produced (see Mollison 1956a)

The exact survival time of fœtal (*e g* cord blood) erythrocytes has been the subject of controversy However their life span appears to be shorter than that of adult cells but to what degree is uncertain (Hollingsworth 1955a Mollison 1956a Gilardi and Miescher 1957)

It seems probable that the normal mechanism of elimination of erythrocytes from the blood stream is by the removal of fragments of cells or of effete intact cells by reticulo endothelial cells in the spleen and elsewhere (Rous and Robertson 1917 Rous 1923) The reticulo endothelial cells probably act passively and remove the cells or fragments in the same way as they remove foreign particles (Miescher 1956 1957) Indirect evidence in favour of the fragmentation hypothesis was provided by Stewart Stewart Izzo and Young (1950) who making use of corpuscles labelled with ^{59}Fe showed that the mechanical fragility of the oldest cells increased before they were eliminated from the circulation Recent work has demonstrated in guinea pigs and rabbits at least that the bone marrow by virtue of its size and rich content of phagocytic cells is the most important organ in physiological erythroclasis (Miescher 1956 Ehrenstein and Lockner 1958) Certainly the normal spleen can be removed without any great effect on the rate of destruction

The changes in the erythrocyte or at its surface which cause the fragmentation or increased sensitivity to mechanical trauma are obscure Presumably actual loss of substance or increased rigidity are the consequence of metabolic failure

It is possible but unproved that influences outside the cell play a part in bringing about the cumulative damage which limits the life of the normal erythrocyte It has been suggested for instance that stagnation of the blood stream particularly in the spleen might be deleterious (Fahraeus 1939 Ham and Castle 1940) and it is conceivable

that tissue lysins normally inhibited by plasma may play a part under conditions of stasis (Londer 1951) Normal plasma too is known to contain potential auto agglutinins and lysins active at 37 C against erythrocytes damaged by enzymes such as trypsin (Rosenthal and Schwartz 1951) or against defective erythrocytes like those of paroxysmal nocturnal hæmoglobinuria It is conceivable that normal corpuscles although apparently insensitive to these agglutinins and lysins in crude tests *in vitro* are significantly affected *in vivo* where they are exposed to the action of these factors for much longer periods of time

The mechanism of hæmolysis where there is a pathological increased rate of cell destruction and the sites in the body where this takes place are considered in later chapters in relation to the different hæmolytic syndromes

Catabolism of Hæmoglobin There seem to be two main channels for the disposal of hæmoglobin liberated by erythrocyte destruction If a cell or a fragment of a cell is taken up by an erythrophage in the bone marrow or spleen or elsewhere in the body (extravascular lysis) the hæm of the hæmoglobin molecule becomes transformed to bilirubin which is eventually eliminated from the circulation by the liver and finally forms a major part of the stercobilinogen of the fæces The iron and protein part of hæmoglobin are retained in the body This is probably the main method by which hæmoglobin is disposed of in health If on the other hand as in certain hæmolytic anæmias the erythrocyte breaks up or is lysed in the blood stream (intravascular lysis) the liberated hæmoglobin is disposed of in two ways if in low or moderate concentration it is retained in the plasma in the form of a haptoglobin hæmoglobin complex if in high concentration part will pass through the renal glomeruli and appear in the urine as hæmoglobinuria and part is quickly broken down in the plasma liberating hæm groups which are oxidized and unite with albumin to form the brown pigment methæmalbumin (Fairley 1941 Allison and ap Rees 1957) The pigment moiety of methæmalbumin is probably excreted by the liver as bilirubin (Pass Schwartz and Watson 1945 London 1950) The haptoglobin hæmoglobin complex is eliminated slowly according to Laurell and Nyman (1957) at a constant rate of about 13 mg per 100 ml per hour It is thought that the complex is taken up by reticulo endothelial cells the end product also being bilirubin (see also p 12)

A detailed consideration of the complicated steps in the break down of bilirubin to fæcal stercobilin is beyond the scope of this book Recent views are to be found in the reviews of Watson (1957) and Billing and Lathe (1958) (see however p 8)

The distinction between an extravascular and an intravascular mechanism of hæmolysis was made as early as 1901 by Hunter Referring to *chronic hæmatocytolysis* (p 363) he said They [the red corpuscles] become spherical deeper in colour and retain their hæmoglobin to the last In this form they continue to circulate until finally they are enclosed within the active cells of the spleen or leucocytes of the blood and are stored up within the *spleen* or in the *capillaries of the liver* [author s italics] He then went on to refer to *acute hæmocytolysis* (p 364) saying The second process is marked by a different series of phenomena The first of these is a liberation of hæmoglobin from the corpuscle It escapes from the corpuscle either alone or in combination with the albuminous stroma Its fate is not as in the former case to be taken up by splenic cells or leucocytes within the blood but it is carried to the liver in the portal blood where it is taken and broken up by the liver cells

EVIDENCE FOR AN INCREASED RATE OF HÆMOLYSIS

As the bile and fæcal pigments are largely derived from the catabolism of hæmoglobin it is natural to expect increased production and elimination of these substances whenever the rate of erythrocyte destruction is increased

Hyperbilirubinæmia In hæmolytic anæmia the plasma bilirubin concentration usually lies between 1 and 3 mg per 100 ml Occasionally it is within the normal range it is rarely above 5 mg per 100 ml The direct Hijmans van den Bergh reaction is usually negative or delayed positive in uncomplicated cases The bilirubin concentration however is an unreliable measure of hæmolysis as it depends not only on the amount of pigment produced but also on the efficiency of the liver in excreting it Moreover the total amount produced depends not only on the rate of hæmolysis but also upon the total number of erythrocytes present For instance the same amount of bilirubin might be expected to be produced per day by the destruction of 5% of a patient s erythrocytes when the total count was 5 000 000 per cu mm as by the destruction of 25% of the erythrocytes when the count was 1 000 000 per cu mm Other things being equal therefore the highest bilirubin levels might be expected in patients with the highest erythrocyte counts In practice however this expected correlation is seldom found as the patients with the highest counts are usually those in whom the rate of hæmolysis is not great *i e* they are patients in whom compensation for hæmolysis is possible (see p 26)

It is probable that in those patients in whom the plasma bilirubin level is normal despite evidence of increased hæmolysis the normal levels are maintained by the ability of the healthy liver

to excrete far more bilirubin than it is normally called upon to do

The relationship one to the other of the bile pigments giving rise respectively to direct and indirect Hijmans van den Bergh reactions has recently been clarified Cole and Lathe (1953) were able to separate by reverse phase chromatography two types of bile pigment neither of which was bound to protein the slow moving less soluble fraction was the normal indirect reacting pigment of the blood stream and the faster moving more soluble fraction was the direct reacting pigment

In a recent review Billing and Lathe (1958) summarize much recent progress The faster moving pigment consists of two fractions I and II both of which predominate in bile and in the blood in obstructive jaundice These fractions are the mono and di glucuronides of prehepatic bilirubin and it is this conjugation which allows the direct Hijmans van den Bergh reaction to proceed Billing and Lathe make the point that the terms indirect bilirubin and direct bilirubin have no real chemical or physiological significance and should be abandoned in favour of bilirubin and conjugated bilirubin

In hæmolytic jaundice bilirubin predominates and only small quantities of pigments I and II are present In hæmolytic disease of the newborn the pigment is entirely composed of bilirubin and it is the inability of the neonatal liver to conjugate bilirubin that is responsible for the physiological jaundice of the newborn and for the very high plasma levels which are reached in hæmolytic disease at that time

The ability of the healthy liver to conjugate and excrete bilirubin is very great According to Billing and Lathe (1958) if all the hæmoglobin in the body were transformed to bilirubin this could be excreted in 10–12 hours It is thus easy to see why patients with hæmolytic anæmia rarely become markedly jaundiced and may in fact not be jaundiced at all According to Crosby (1955) it nevertheless takes about $1\frac{1}{2}$ hours for plasma to be completely cleared of bilirubin present at a normal concentration This figure is arrived at in the following way at a concentration of 0 5 mg per 100 ml there would be 15 mg of bilirubin circulating in a plasma volume of 3 000 ml if the total amount of bilirubin leaving the blood stream in 24 hours was 250 mg then each 15 mg would be excreted in $\frac{24 \times 15}{250}$ hours $= 1\frac{1}{2}$ hours approximately

Excretion of Urobilinogen

Urobilinogen is the name given to the fæcal pigments derived from bilirubin which when reduced give coloured compounds with Ehrlich s reagent dimethylaminobenzaldehyde In the

fæces they exist as a series of colourless compounds *d* urobilinogen *i* urobilinogen (mesobilirubinogen) and *l* urobilinogen (stercobilinogen) and as orange yellow derivatives formed as the result of loss of two hydrogen atoms *d* urobilin *i* urobilin and *l* urobilin (stercobilin) (Watson 1957)

In hæmolytic anæmia the excretion of fæcal urobilinogen is often far in excess of normal and the quantitative estimation of the pigments has often been used as a measure of the degree of increased hæmolysis (Watson 1938 Crosby and Akeroyd 1952) In the following section the accuracy of such estimations their interpretation and the figures obtained in health will be briefly considered

Normal Urobilinogen Excretion One gram of hæmoglobin theoretically should give rise on degradation to approximately 35 mg of urobilinogen This relationship is based on the ratio of the molecular weights of hæmoglobin (68 000) and four molecules of hæm (2 000) from which the bilirubin and urobilinogen are derived If therefore in a normal adult 6 *g* of hæmoglobin are catabolized daily this should give rise to 210 mg of urobilinogen

The figure of 6 g is arrived at as follows A 70 kg man with an erythrocyte volume of 30 ml per kg will have a total circulating erythrocyte volume of 2 100 ml Assuming the MCHC to be 33% this means that the total circulating hæmoglobin is about 700 g Dividing this figure by 120 on the assumption that 1/120th of the hæmoglobin is catabolized daily gives a figure of approximately 6 g

Not all the fæcal urobilinogen comes from catabolized hæmoglobin Studies with ^{15}N labelled glycine have shown that a significant proportion of the fæcal hæm pigment is derived from sources other than hæmoglobin (London West Shemin and Rittenburg 1950 Gray Neuberger and Sneath 1950 Watson 1957) In health this proportion may be as high as 10–20% in pernicious anæmia it is higher and may reach 40% (London and West 1950) It is thought that the non hæmoglobin derived pigment comes from several sources (*a*) from tetrapyrrol (hæm or porphyrin) pigments formed but not utilized for hæmoglobin formation (*b*) from hæmoglobin containing cells destroyed in the bone marrow before delivery into the blood stream (*c*) from defective very short lived cells possibly trapped and destroyed in the spleen or (*d*) from myoglobin and other hæm pigments such as catalase (*a*) (*b*) and (*c*) represent early appearing urobilinogen

Returning to the urobilinogen excretion of the healthy 70 kg adult referred to above 30 mg of pigment should be added to the 210 mg of hæmoglobin derived pigment to allow for the early appearing pigment This gives a total of 240 mg of pigment excreted per day

Study of published data on urobilinogen excretion in health shows that figures as high as this are rarely attained and that the recorded normal ranges are very wide Greppi (1926) gave a range of 90–150 mg per day Watson and Bilden (1941) 40–280 mg per day Watson (1942) 40 250 mg per day and Maclagan (1946) 22–121 mg per day Sparkman (1939) recorded figures varying from 76–590 mg per 100 g of fæces as the daily excretion in 100 normal adults Mills and Mason (1952) gave normal values for children

✓ It is obvious that the daily urobilinogen excretion of a healthy child will be less than that of an adult because his total erythrocyte volume is much less and the same is true of an anæmic adult if the rate of hæmoglobin breakdown is normal The only satisfactory way to get round this difficulty is to relate the pigment excretion to the total circulating hæmoglobin (Greppi 1926 Watson 1938)

Greppi used a hæmolytic index in which he related the patient s weight hæmoglobin percentage and urobilinogen excretion to normal values of 70 kg 100% and 120 mg per day respectively This index normally 1.0 gave values as high as 10–15 in acute hæmolytic states Miller Singer and Dameshek (1942) reported urobilinogen excretion in relation to the total circulating hæmoglobin and gave values of 11–21 mg per 100 g of hæmoglobin in health while Giblett and her co workers (1956) using a similar index reported an excretion of 0.14–0.48 mg per g of hæmoglobin (mean 0.248 mg) in 18 healthy young men Again the excretion figures vary widely even when based on the total circulating hæmoglobin and there is a disquieting discrepancy between the last two sets of data quoted the mean of those of Giblett and her co workers exceeding the upper limit of normal given by Miller Singer and Dameshek

It is obvious that it is difficult to estimate fæcal urobilinogen with any degree of accuracy The technical difficulty of the collection of 24 hour or 96 hour samples of fæces difficulties in obtaining representative samples of the specimens and the use of an arbitrary colour standard in the actual estimation all combine to reduce the reliability of the figures obtained Furthermore constipation diarrhœa and antibiotics reduce the amount of pigment that can be estimated

✓ As already mentioned not all the fæcal urobilinogen is derived from the hæmoglobin of effete erythrocytes contrariwise not all the catabolized hæmoglobin can be accounted for as fæcal or urinary urobilinogen Although in dogs with artificial biliary fistulæ

given acetylphenylhydrazine 88% of the hæm liberated from the breakdown of hæmoglobin could be recovered as bilirubin in the bile (Cruz Hawkins and Whipple 1942) the proportion recoverable in man is unknown *Moreover it is known that the amount of pigment* that can be estimated as fæcal urobilinogen is considerably less than the bilirubin excretion This suggests that either the conversion of bilirubin to urobilinogen is not quantitative or else that the urobilinogen is altered in part to other substances which are not readily estimated or that some of the pigment is reabsorbed (Watson 1942 Crosby and Akeroyd 1952 Gray 1953)

The discrepancy between hæmoglobin destruction and urobilinogen excretion is considered in some detail by Watson James (1955) who studied the excretion of two normal males over many months and Watson (1957)

In Watson s view the discrepancy does not seem to be accountable by the formation of dipyrrolmethenes such as mesobilirubifuscin which although a normal constituent of the fæces he considers to be a by product of hæm synthesis Watson referred to a patient with refractory anæmia whose average urobilinogen excretion was 30 mg daily although a figure of 133 mg would be expected on the basis of hæmoglobin catabolism An increase in mesobilirubifuscin or failure of conjugation of bilirubin was ruled out as causes and conservation of pigment and its re utilization for hæm synthesis also seemed improbable He concluded that reabsorption of pigment to an abnormal degree without re utilization might have been the explanation That some urobilinogen is normally reabsorbed is demonstrated by the presence of the pigment in the urine The amount however excreted daily in health is small < 3.5 mg (Watson 1942) and the greater part of that absorbed is thought to be re excreted by the liver

Urobilinogen Excretion in the Fæces in Hæmolytic Anæmia That the fæces of patients with hæmolytic anæmia are often dark in colour and their content of urobilinogen unusually high have been known for many years (see Watson 1937 1938) Crosby and Akeroyd (1952) and Crosby (1955) have brought the subject up to date In an adult the fæcal urobilinogen excretion often exceeds 500 mg per day and occasionally rises to within the 1 000–1 500 mg range A patient with a chronic hæmolytic anæmia whose erythrocyte count is in equilibrium as the result of the maximum possible hyperplasia of the bone marrow can synthesize 6–8 times the normal amount of hæmoglobin (Crosby and Akeroyd 1952) namely about 50 g daily compared with the normal synthesis of 6–7 g in a 70 kg man A daily breakdown of 50 g of hæmoglobin would theoretically be expected to yield *1 750 mg of urobilinogen However* as has been explained above this amount of pigment is not likely to be estimable

Crosby (1955) has given details of two patients intensively studied over long periods of time In one of these patients who had a hereditary non spherocytic hæmolytic anæmia the output of pigment based on

4 day averages over a period of 2 months ranged between 400 and 2 000 mg per day The anticipated value was 1 800 mg daily This patient s excretion varied in a remarkable 25-day cyclic manner which could not be explained on any variation in the rate of hæmolysis Another patient with paroxysmal nocturnal hæmoglobinuria similarly studied had a daily output of urobilinogen ranging from 90 to 340 mg per day The estimated theoretical output in this patient whose disease was in a stable phase was 500 mg daily

Hagen and MacDonald (1954) give comparable data in four patients with acquired hæmolytic anæmia the recovery of urobilinogen in the fæces was 79 87 51 and 56 % of the theoretical estimated total in a patient with paroxysmal nocturnal hæmoglobinuria it was 41% and in two patients with hereditary spherocytosis 113% and 57%

Baldini and di Pietrantoni (1957) have carried out similar studies in eight patients with hæmolytic anæmia. The discrepancy between the calculated urobilinogen excretion and that actually estimated varied from 0 to 65 in five of them more than 50% of the pigment was unaccounted for

Urobilinogen in the Urine in Hæmolytic Anæmia A darkening of the urine particularly on standing due to excess of urobilin is frequently found in cases of hæmolytic anæmia The quantitative estimation of the pigment cannot however be used as a reliable index of erythrocyte destruction Urobilinuria is an indication that the liver is unable to re excrete urobilinogen reabsorbed from the bowel rather than a sign of increased hæmolysis (Watson 1937 Barker 1938)

In uncomplicated hæmolytic anæmia the daily excretion may amount to 5–20 mg Crosby and Akeroyd (1952) made the interesting observation that when the fæcal pigment excretion of one of their intensively studied patients fell the urinary excretion increased This suggests that the fall in fæcal excretion might have been accounted for by increased absorption

Hæmoglobinæmia and Hæmoglobinuria

In normal plasma there is very little free hæmoglobin probably less than 4 mg per 100 ml (Crosby and Dameshek 1951) In those types of hæmolytic anæmia in which hæmolysis takes place predominantly in the blood stream the plasma hæmoglobin may rise to 100–200 mg per 100 ml or even more In such cases hæmoglobinuria develops and the loss of pigment in this way may account for a substantial proportion of the total pigment excretion

The earlier work on hæmoglobinuria was considered at length in Yule s (1942) review and more recently by Ham (1955) Yule concluded that hæmoglobin probably passed through the glomeruli even at low plasma concentrations only to be reabsorbed by the renal tubules

It was postulated that this mechanism led to a renal threshold phenomenon for it was only when the plasma concentration exceeded approximately 135 mg per 100 ml that overt hæmoglobinuria developed (The well known occurrence of a lowering of the renal threshold in chronic intravascular hæmolysis was attributed to an overloading of the renal tubular cells with reabsorbed pigment and impairment of their capacity for further absorption) (Lichty Havill and Whipple 1932 Gilligan Altschule and Katersky 1941) Gilligan Altschule and Katersky also demonstrated that the rate of clearance of hæmoglobin from the plasma was directly related to the plasma hæmoglobin level

New facts have demonstrated more precisely the way in which hæmoglobin is transported in the plasma and have provided a different interpretation of the renal threshold phenomenon

(It is now known that there exist in the plasma several proteins termed haptoglobins which have the property of combining with hæmoglobin liberated into the plasma (Jayle and Boussier 1955) On electrophoresis in a Tiselius apparatus or on filter paper the haptoglobins separate with the α globulins According to Jayle Boussier and Tonnelat (1956) each molecule of haptoglobin can combine with one or two molecules of hæmoglobin)

Smithies (1955a b) has demonstrated that when electrophoresis *of serum is carried out in starch gels three different hereditary patterns* of hæmoglobin haptoglobin complexes can be distinguished Smithies also reported that the haptoglobins could normally bind up to about 125 mg of hæmoglobin per 100 ml plasma any hæmoglobin in excess of this remaining uncombined

Laurell and Nyman (1957) injected hæmoglobin intravenously into normal subjects in an amount calculated to saturate the plasma haptoglobins The hæmoglobin haptoglobin complex disappeared *steadily at a rate of about 13 mg per 100 ml per hour and it took up* to a week for the haptoglobins to be fully regenerated Laurell and Nyman concluded that hæmoglobin could not exist free in the plasma unless the binding capacity of the plasma was fully saturated and that the minimum amount of hæmoglobin injected intravenously which would result in hæmoglobinuria would vary with the amount of circulating haptoglobin They estimated that the amount of hæmoglobin which may be bound by the plasma of normal subjects varies from 50–140 mg per 100 ml According to these Swedish workers the renal threshold for hæmoglobin depends therefore on the ability of the haptoglobins of the plasma to bind hæmoglobin as well as upon the ability of the renal tubular cells to reabsorb hæmoglobin from the glomerular filtrate The reduction in renal threshold in the presence of chronic intravascular hæmolysis or following repeated intravenous injections of hæmoglobin (Lichty Havill and Whipple 1932) could be explained at least in part on the slow regeneration of plasma haptoglobin) Laurell and Nyman mentioned that sera from some patients with acquired hæmolytic anæmia or untreated pernicious anæmia contained no demonstrable haptoglobin and they referred back to the early work of Sellards and Minot (1916) who reported that less intravenously administered hæmoglobin was required to produce hæmoglobinuria in patients with pernicious anæmia or hæmolytic anæmia than in health

Allison and ap Rees (1957) have also published interesting observations on the relationship between the plasma haptoglobins, renal threshold and hæmoglobinuria. Also using starch-gel electrophoresis they confirmed that the great majority of subjects fall into one of three groups: 1-1 with only one haptoglobin migrating in the fast α_2 position, 2-2 with three haptoglobins migrating as three distinct bands in the $\alpha\beta$ region, and 1-2 with four haptoglobins, three migrating slightly faster than those of group 2-2 plus one which is apparently the same as that of group 1-1. Allison and ap Rees also reported that umbilical cord blood was distinctive in not containing demonstrable haptoglobins and that a small percentage of older children and adults have sera either lacking haptoglobins or at the most containing a single component giving a band in the $\alpha\beta$ region.

Allison and ap Rees also added oxyhæmoglobin to serum *in vitro*. When more than 100–175 mg per 100 ml were added free oxyhæmoglobin could be demonstrated migrating as a band distinct from the hæmoglobin-haptoglobin complexes as well as methæmalbumin which migrated in the albumin region. They too concluded that the renal threshold for hæmoglobin depends primarily on the haptoglobin level of the plasma. Several patients suffering from intravascular hæmolysis were studied. In four patients with paroxysmal nocturnal hæmoglobinuria and in two patients with paroxysmal cold hæmoglobinuria haptoglobins were absent or present in quantities too small to be detected, and in one case of march hæmoglobinuria the haptoglobin concentration was less than half the normal value. In one possible case of paroxysmal nocturnal hæmoglobinuria, examined at least 8 years after apparent complete recovery, the haptoglobins were normal, as they were also in a patient with paroxysmal cold hæmoglobinuria, who had a single attack several years previously. Allison and ap Rees pointed out that the low haptoglobin levels of these patients could be the result of either diminished formation, for genetic or other reasons, or rapid removal from the blood stream as the result of the continuing intravascular hæmolysis. The second hypothesis seems the more likely on the available evidence.

Aber, Neale and Northam (1957) and Neale, Aber and Northam (1958) studied, using simple paper electrophoresis, the binding of hæmoglobin by serum *in vitro* with particular reference to megaloblastic anæmia and hæmolytic anæmia. They also have concluded that the serum proteins capable of binding hæmoglobin are lost from the circulation in the process of clearing the plasma of hæmoglobin. Using paper electrophoresis Aber, Neale and Northam have found that a normal serum will bind hæmoglobin to α_2 globulin, β_1 globulin and albumin in that order, and that following a hæmolytic episode the β_1 globulin binding component returns first and then the α_2 globulin. They concluded that the behaviour of serum in respect of binding hæmoglobin when hæmoglobin is added at a concentration of 30 mg per 100 ml provides important evidence as to whether intravascular hæmolysis is occurring or has recently taken place.

Brus and Lewis (1959) have more recently reported on the results of a survey of a large number of patients with various types of hæmolytic anæmia. Measurement of erythrocyte survival by the radiochromium method and the demonstration of haptoglobins using cellulose acetate

paper electrophoresis showed that a hæmoglobin turnover exceeding twice the normal was usually accompanied by disappearance of the haptoglobins Several patients however were studied in whom the erythrocyte life span was reduced to one third or one quarter of the normal without the haptoglobins disappearing These patients were anæmic and in each instance the total hæmoglobin catabolized daily was less than twice the normal In other patients an abnormally high level of plasma globulins was associated with demonstrable haptoglobins although the hæmoglobin turnover exceeded twice the normal rate Steroid therapy likewise caused a reappearance of haptoglobins despite continuing hæmolysis

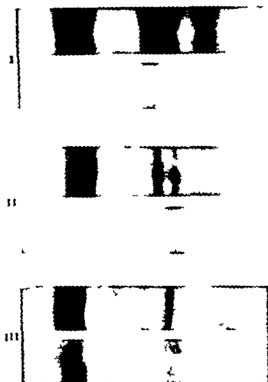
This study demonstrates clearly that testing for the presence or absence of haptoglobins is a sensitive method of detecting hæmolysis However it also shows that the result may be affected by factors other than the rate of hæmolysis These have to be kept in mind when assessing the value of any particular test

The findings in health in hereditary spherocytosis and in paroxysmal nocturnal hæmoglobinuria are compared in Fig 1

The genetics of the haptoglobins have proved to be of great interest Two genes appear to control groups 1–1 and 2–2 haptoglobins respectively while group 2–1 represents the heterozygous state Ultracentrifugal studies have shown that group 1–1 haptoglobins when homozygous are $S_{20\,w}6$ proteins while group 2–2 are $S_{20\,w}11$ group 2–1 haptoglobins are formed of three components $S_{20\,w}6$ and $S_{20\,w}11$ as well as a major component $S_{20\,w}9$ not present in the homozygotes (Bearn and Franklin 1958)

The incidence and severity of hæmoglobinæmia in the hæmolytic anæmias were reviewed by Crosby and Dameshek (1951) In addition to finding high values in the well known types of hæmoglobinuria they reported slightly raised concentrations in several types of acquired hæmolytic anæmia (without hæmoglobinuria) but not in hereditary spherocytosis in overt sickle cell disease but not in sickle cell trait and in severe Mediterranean anæmia (thalassæmia major) but not in the trait (thalassæmia minor) It must be emphasized that the presence of excess hæmoglobin in the plasma is only a reliable sign of hæmolysis if the observer can be sure that the lysis has not been caused during or after the withdrawal of the blood

Methæmalbuminæmia Methæmalbumin was first observed by Fairley and Bromfield (1934) in the plasma of a patient suffering from blackwater fever The plasma was brownish in colour and spectroscopic examination showed an absorption band in the red part of the spectrum (at 624 mμ) Subsequently Fairley (1941) showed that methæmalbumin could be detected in the plasma in several types of hæmolytic anæmia with hæmoglobinuria when hæmolysis was taking place within the blood stream *e g* in blackwater fever in paroxysmal nocturnal hæmoglobinuria and



Fi 1 Demon tration of serum h ptogl bin

I N rmal serum Hapt globins are present a shown by th b nd of added hemogl bin (0 3 mg per ml) appea ing in the a gl bul n position (l wer lectroph r is strip)

II Serum f om a case of here litary sph er cytosi   H ptogl b n are absent The b nd of add d hemogl bin (0 3 mg per ml) has a mobility correspon ling with th t of β glob lin (low el trophore i strip)

III Serum from a case of parox ys mal n ctu nal hemoglobinuri N h m gl bin has been added Free hem gl bin is present m gr ting with th β gl b lin and al m th m lb mi migrating with albumin

Cellulose acetate p per electroph resi   3 hours at 1 0 140 v and 1 ma per strip The strip were d v i d l ngitu l n lly the upper part wa staine l with Ponceau S t demon tr te se um protei   the lower part was stained w th leuco malachite green and hydrogen pe oxide to demon trate h m compoun ls (Hrus and Lewis 1))

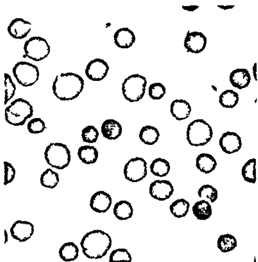


FIG. 2. Photomicrograph of a blood film of a patient suffering from idiopathic acquired hemolytic anæmia (warm antibody type) (Case 11 of Dacie 1954). The contrast between the darkly staining spherocytes and the polychromatic macrocytes is well shown. × 700.

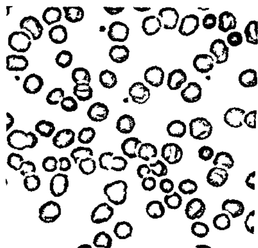


FIG. 3. Photomicrograph of a blood film of a patient of blood group A who received a transfusion of group O blood containing high titre immune anti-A. The small microspherocytes, many of them in pairs stuck together, represent some of the recipient's corpuscles which have been damaged by the anti-A. × 700.

in *Cl welchii* septicæmia In addition he found the pigment was present in smaller amounts in the plasma of patients with hæmolytic anæmia of unknown origin without hæmoglobinuria and in three patients suffering from typical severe pernicious anæmia On the other hand tests for the pigment were negative in two patients with hereditary spherocytosis and only very weakly positive in a third

Methæmalbumin is distinct chemically and spectroscopically from methæmoglobin (absorption band at 630 mμ) It is a hæmatin albumin compound and is formed as a result of the degradation of hæmoglobin liberated into plasma Methæm albumin if not detectable by the position of its characteristic but rather faint absorption band in the red can be demonstrated by covering the serum or plasma with ether and then adding a one-tenth volume of concentrated ammonium sulphide This results in the formation of a hæmochromogen with a relatively intense sharply defined α absorption band at 558 mμ (Schumm s test)

Fairley s work is now generally accepted and the presence of methæmalbumin demonstrated in serum by spectroscopy or by a positive Schumm s test is probably an indication of a major degree of intravascular hæmolysis Schumm s test is certainly positive in some cases of acquired hæmolytic anæmia pernicious anæmia and hæmolytic disease of the newborn as well as in the hæmoglobinurias However no extensive series of cases seems to have been studied from this point of view and the whole subject of hæm pigments in the plasma in blood diseases merits re investigation in view of the new knowledge of the role of the haptoglobins (see p 12)

Aber Neale and Northam (1957) and Neale Aber and Northam (1958) have however recently described an in vitro test which they believe provides a simple means of determining whether hæmolysis is taking place or has occurred recently According to them Schumm s test is negative as long as the hæmoglobin can be taken up by the haptoglobins When the α_2 haptoglobin is deficient hæmoglobin added to serum *in vitro* is then taken up by a β_1 globulin as demonstrated by paper electrophoresis Such sera give after the addition of ammonium sulphide a hæmochromogen with an absorption band at 558 mμ The band however is apparently developed more slowly than that derived from methæmalbumin under the same circumstances

Hæmosiderinuria The presence of brownish granules in the urine giving Perls s reaction for free ferric iron is characteristic of the chronic hæmoglobinurias The iron is derived from

hæmoglobin absorbed from the glomerular filtrate and subsequently broken down within the renal tubular cells As long ago as 1911 Marchiafava and Nazari recognized a granular form of hæmoglobin in the urine of a case of paroxysmal nocturnal hæmoglobinuria Rous (1918) observed hæmosiderin in the urine of a patient with acquired hæmolytic anæmia and in the urine of several patients suffering from pernicious anæmia who had received many transfusions Later Marchiafava (1928) referred to paroxysmal nocturnal hæmoglobinuria as Anemia emolitica con emosiderinuria perpetua Perpetual hæmosiderinuria is in fact a reliable sign of clinically important chronic intravascular hæmolysis for the urine will be found to contain iron granules even if there is no hæmoglobinuria at the time Hæmosiderin is not however found in the urine at the first onset of a hæmolytic attack even if accompanied by hæmoglobinuria as the pigment has to be absorbed by the tubular cells of the kidney and re excreted a process which occupies several days at least (Yule 1942)

The incidence and significance of hæmosiderinuria were re investigated by Crosby and Dameshek (1951) They found hæmosiderin in the urine of every patient whose plasma continuously contained abnormal amounts of hæmoglobin and observed in general a parallelism between the amount of urinary hæmosiderin and the degree to which the plasma hæmoglobin level was raised *Only very small amounts were found in the urine of* patients whose plasma hæmoglobin levels were less than 20 mg per 100 ml on the other hand with plasma hæmoglobin levels greater than 40 mg the amount of hæmosiderin was as a rule sufficient to give a visible Prussian blue colouration to the urinary deposit when Perls s reaction was carried out

Other Signs of Increased Hæmolysis

Another sign of excess hæmolysis is an increased formation of endogenous carbon monoxide (Engstedt 1957) Carbon monoxide is probably derived from the α methene bridge during the catabolism of hæm Engstedt measured its concentration in alveolar air and from this figure calculated the concentration of carboxyhæmoglobin in the blood He found a highly significant correlation between the rate of hæmolysis calculated from carboxyhæmoglobin values and those obtained from fæcal pigment excretion erythrocyte life span measurements using ^{51}Cr and the height of the reticulocyte count A further ingenious method of demonstrating excess hæmolysis was suggested by Robinson (1950) who in a preliminary communication showed that the urine of two patients suffering from intravascular hæmolysis contained relatively large amounts of carbonic anhydrase derived from broken down erythrocytes

CLINICAL FINDINGS IN INCREASED HÆMOLYSIS

The clinical findings in each of the more important hæmolytic syndromes will be given in subsequent chapters The main clinical signs are however summarized in the three following paragraphs

The familiar triad of anæmia acholuric jaundice and splenomegaly is certainly suggestive of a hæmolytic anæmia but one or more signs may be absent and sometimes all three On the other extreme anæmia may be profound in degree and lead to prostration dyspnœa cardiomegaly with a raised cardiac output and hyperdynamic circulation and tachycardia The jaundice of increased hæmolysis is rarely marked except in the neonatal period The urine is usually dark because of an increased content of urobilin it rarely however contains bile pigment Hæmoglobinuria may cause the urine to be burgundy or porter coloured or even almost black The stools are also typically darker than normal in colour

The spleen is usually palpable but it seldom extends as far down as the umbilicus The liver is often palpable below the costal margin in the more anæmic patients but its enlargement does not often extend further than 5–7 cm The superficial lymph nodes are sometimes slightly enlarged

Other signs which may be associated with increased hæmolysis are ulcers on the legs above the ankles in the absence of varicose veins and Raynaud s phenomena Finally markedly anæmic patients usually have a moderate degree of fever The symptoms as opposed to the signs associated with a hæmolytic anæmia are too varied to be summarized

THE BLOOD PICTURE IN HÆMOLYTIC ANÆMIA

Evidence of Hæmolysis Important evidence of increased blood destruction may be obtained by examination of the blood itself Certain (pre hæmolytic) abnormalities of the erythrocytes are probably almost always associated with an increased rate of *hæmolysis The most important of these abnormalities are spherocytosis* and *schistocytosis* (fragmentation)

Spherocytosis

Spherocytes are erythrocytes which are more nearly spheroidal and less distinctly disc like than are normal cells It should be emphasized that all grades of spherocytosis are met with ranging from cells which retain their biconcavities but whose thickness

hæmoglobin absorbed from the glomerular filtrate and subsequently broken down within the renal tubular cells As long ago as 1911 Marchiafava and Nazari recognized a granular form of hæmoglobin in the urine of a case of paroxysmal nocturnal hæmoglobinuria Rous (1918) observed hæmosiderin in the urine of a patient with acquired hæmolytic anæmia and in the urine of several patients suffering from pernicious anæmia who had received many transfusions Later Marchiafava (1928) referred to paroxysmal nocturnal hæmoglobinuria as 'Anemia emolitica con emosiderinuria perpetua Perpetual hæmosiderinuria is in fact a reliable sign of clinically important chronic intravascular hæmolysis for the urine will be found to contain iron granules even if there is no hæmoglobinuria at the time Hæmosiderin is not however found in the urine at the first onset of a hæmolytic attack even if accompanied by hæmoglobinuria as the pigment has to be absorbed by the tubular cells of the kidney and re excreted a process which occupies several days at least (Yuile 1942)

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is probably not reversible and complete spherocytosis is rarely seen. In addition, spherical forms are not normally "fragile" to hypotonic saline (Ponder, [illegible] Gillespie [illegible]) [illegible] spherocytes are

The association between spherocytosis and increased fragility to hypotonic saline [illegible] is well [illegible] 1934, Castle and Daland 1937, Guest 1948, Crosby 1952). The more nearly spherical a cell, the less water it can absorb from hypotonic media without [illegible] its maximum surface. Hence spherocytes will [illegible] in media of a lesser degree of hypotonicity than do normal, more discoidal cells of the same volume. Castle and Daland (1937) and Guest (1948) showed that spherocytes from cases of hereditary spherocytosis and normal corpuscles swell to a similar degree when placed in hypotonic media. Spherocytes do not lyse because they swell more than do normal corpuscles in a hypotonic solution; they lyse because their ability to swell is limited by their already spheroidal shape. Osmotically both types of cell behave in the same way. Crosby (1952) gives the average dimensions of normal erythrocytes as well as of several different types of pathological cells, including the "hereditary spherocyte" (see below). He stressed that it is the reduction in surface area/volume ratio which is the essence of spherocytosis (a sphere having the least possible surface area for its volume).

Types of Spherocytosis Apart from spherical forms there are two main classes of spherocytes: *hereditary (congenital) types*, e.g., those of hereditary spherocytosis, and *acquired types* due not to a congenital defect but to damage to the erythrocytes caused by a variety of harmful agents such as chemicals, haemolytic immune antibodies, or the effects of heating, etc. The morphological changes caused by these various processes are similar or perhaps identical. Functionally each type represents a prehaemolytic change; in each type, too, there is an irreversible reduction in surface area resulting in increased osmotic fragility.

The Hereditary (Congenital) Spherocyte Naegeli (1923) recognized the increased spheroidicity of the microcytic erythrocytes of hereditary spherocytosis and referred to them as "Kugelzellen" (globe cells); later he introduced the term "Sphärocyte" (spherocyte) (Naegeli 1931). Naegeli's spherocytes were soon generally considered to be pathognomonic of hereditary spherocytosis. Although this conception was erroneous, spherocytosis is certainly a very characteristic sign of the hereditary disease (see p. 115). The spherocytic change is a dynamic one. It is not a question of a fixed inherited abnormality of shape; it is

or breadth is increased to cells which are almost spherical As a rule the volume of a spherocyte is normal (see Table 1 p 32) hence any increase in thickness or breadth of the cell must be associated with a diminution in diameter Spherocytes are thus usually correctly called microspherocytes In stained blood films they appear as small usually perfectly round cells staining relatively intensely with Romanowsky dyes and usually showing no central pallor (Fig 2) Their unusual rotundity can also be recognized in ' wet preparations of fresh blood The increased density of staining is due not only to their shape but also to a slightly increased concentration of hæmoglobin (Gaffney 1957 see also Table 1 and p 104)

According to Crosby (19..) the term spherocyte was first used by Christophers and Bentley in a monograph on blackwater fever published in India in 1908 Hunter (1901) had referred to the presence in pernicious anæmia of so called microcytes — small spherical deeply coloured corpuscles resembling minute red blood corpuscles He added (p 67) that similar bodies can be produced artificially by action of destructive agents

Spherocytes have to be differentiated from *spherical forms* According to Ponder (1948) spherical forms were first observed by Hamburger who in 1895 noted that when mammalian erythrocytes were suspended in saline or sugar containing media they appeared not as discs but as spheres This change can be reversed by the addition of plasma or serum to the suspension Furchgott (1940) showed that the sphering change was facilitated by the increase in pH which occurs when a thin layer of a cell suspension is placed between two glass surfaces as in ordinary microscopy and by the adsorption to glass of an anti sphering substance normally present in plasma Furchgott and Ponder (1940) showed that this substance was an albumin (According to Trotter (1956) sphering between slide and cover glass is due to the glass surfaces being contaminated with lipid of unknown composition (but possibly a fatty acid) derived from human skin as a consequence of handling the glass)

The stages in disc sphere transformation have been described by Ponder (1948) as disc crenated disc crenated sphere finely crenated sphere and sphere This sequence of events is also brought about by a wide range of hæmolytic agents including saponin brilliant green and amboceptor-complement in sublytic concentrations (Ponder 1948) In lytic concentrations the lysins cause the cells to become prolytic spheres and finally to fade from view as hæmolysis proceeds The sequence of changes as it occurs in saline suspensions of erythrocytes kept between glass surfaces is reversible As a cell becomes more and more nearly spherical its surface becomes puckered—hence the crenated appearance—and eventually when the cell is spherical presumably thickened When the process is reversed the thickenin and crenations disappear and the normal condition of the cell surface is restored

Spherocytes differ from *spherical forms* in several important ways the change to spheroidicity is *not* preceded by crenation the process

is probably *not* reversible and complete sphering is rarely seen In addition spherical forms are not typically fragile to hypotonic saline (Ponder 1937 Gillespie 1943) spherocytes are

The association between spherocytosis and increased fragility to hypotonic saline (osmotic fragility) is well known (Haden 1934 Castle and Daland 1937 Guest 1948 Crosby 1952) The more nearly spheroidal a cell the less water it can absorb from hypotonic media without stretching its inextensible surface Hence spherocytes will undergo lysis in media of a lesser degree of hypotonicity than do normal more discoidal cells of the same volume Castle and Daland (1937) and Guest (1948) showed that spherocytes from cases of hereditary spherocytosis and normal corpuscles swell to a similar degree when placed in hypotonic media Spherocytes do not lyse because they swell more than do normal corpuscles in a hypotonic solution they lyse because their ability to swell is limited by their already spheroidal shape Osmometrically both types of cell behave in the same way Crosby (1952) gives the average dimensions of normal erythrocytes as well as of several different types of pathological cells including the 'hereditary spherocyte (see below) He stressed that it is the reduction in surface area volume ratio which is the essence of spherocytosis (a sphere having the least possible surface area for its volume)

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rather that the erythrocytes undergo in the course of their lifetime a progressive and striking increase in their thickness and at the same time undergo a diminution in diameter The nucleated erythrocyte precursors in the bone marrow of patients with hereditary spherocytosis are not abnormal The reticulocytes too are discoidal in shape although they may be slightly less discoidal than normal reticulocytes (Paolino 1949) It is later when circulating in the blood stream and probably particularly within the spleen that the cells become progressively more spherocytic Crosby (1952) suggested that an undue loss of surface lipid might be correlated with (? cause) the shrinkage in surface area)

As discussed on p 133 the basis of the disorder hereditary spherocytosis seems to be a genetically determined metabolic defect of the erythrocytes involving carbohydrate and phosphorus metabolism The spherocytosis is probably the consequence of the defect but the exact way in which the change is bought about still remains obscure

Hereditary spherocytes undergo autohæmolysis *in vitro more rapidly than do normal corpuscles when kept under sterile* conditions (Dacie 1941 Caroli Etévé Parof and Robineau 1949 Young Izzo and Platzer 1951 Selwyn and Dacie 1954 Young *et al* 1956) After 48 hours incubation ten times the normal amount of hæmolysis may take place The exact cause of the increased rate of hæmolysis is obscure but it is presumably dependent on the metabolic defect or handicap from which hereditary spherocytes suffer Selwyn and Dacie (1954) showed as with normal corpuscles that the hæmolysis of hereditary sphero cytes is markedly reduced if glucose is added to the blood in sufficient amount to prevent the glucose concentration from falling below 100 mg per 100 ml during the period of incubation The rate of hæmolysis however is not restored to normal by the addition of glucose The rapid rate of autohæmolysis is associated with an unusually marked increase in osmotic fragility on incubation Further details are given on p 101

Bittorf (1914) and Dacie (1949) demonstrated that hereditary spherocytes were more easily hæmolysed by reduction in pH than were normal corpuscles The increase in acid fragility like the increase in osmotic fragility is probably due to the spherocytes being unable to swell in an acid medium to the same extent as can normal corpuscles (The effect of pH and hypotonicity on the cell volume of normal red cells was described in detail by Hampson and Maizels (1926–27))

Other Types of Hereditary Spherocytes The spherocytes of hereditary spherocytosis are typically and conspicuously round

in contour (Fig 37 p 94) Occasionally spherocytes which tend to be oval or irregular in shape are observed Examples of this sort were recorded by Wyandt Bancroft and Winship (1941) in the blood of a boy both of whose parents were carrying the trait for hereditary elliptocytosis and by Holst Larsen (1947) who published details of eleven cases of hereditary elliptocytosis in one family In seven of Holst Larsen's patients there was evidence of anæmia the elliptocytic erythrocytes were admixed with small rounded and irregularly shaped microspherocytes in the more anæmic patients Another patient in whose blood numerous very small irregularly shaped microspherocytes were conspicuous in films after splenectomy (Fig 54 p 160) was recorded by Dacie Mollison Richardson Selwyn and Shapiro (1953 Case 11) A blood film showing more regular oval microspherocytes is illustrated in Fig 64 (p 164)

The 'Acquired' Spherocyte As already indicated spherocytes morphologically identical with those of hereditary spherocytosis *i.e* rounded microspherocytes are frequently conspicuous in the blood of patients suffering from a variety of hæmolytic disorders They are for instance found in acquired hæmolytic anæmias associated with auto antibody formation (Fig 2 p 15) and spherocytes of apparently similar type may be readily produced experimentally in animals by the administration of heterospecific hæmolytic sera (see Chapter 11) Erythrocytes which have adsorbed immune anti A or immune anti B may also become spherocytic This change has been observed following the transfusion to group A patients of group O plasma (Ervin and Young 1950 Ervin Christian and Young 1950) (Fig 3) and in hæmolytic anæmia of the newborn where the mother is group O and the child group A (Grumbach and Gasser 1948 Crawford Cutbush and Mollison 1953) (In each case the spherocytosis appears to be due to damage to the cell surfaces causing irreversible shrinkage) The exact mechanism of the change however remains obscure The relationship between the production of spherocytosis and the character and specificity of the antibody is discussed in Chapter 11

The osmotic and mechanical fragilities of the above types of acquired spherocytes are increased whether or not they swell to the same extent as hereditary spherocytes in hypotonic media is uncertain On incubation at 37° C *in vitro* acquired spherocytes undergo a variable and sometimes increased rate of autohæmolysis (Dacie 1950a Selwyn and Dacie 1954 Young *et al* 1956)

Spherocytes of similar morphology have been observed in

hæmolytic anæmias due to drug sensitivity *e g* sulphonamide hæmolytic anæmia (Gilligan and Kapnick 1941, Ross and Paegel 1946 see Chapter 15) A mild to moderate degree of spherocytosis is not uncommon in chronic myeloid leukæmia and myelosclerosis The significance of the change is, however, not yet understood Spherocytosis is also fairly regularly found after severe burns (*Fig* 4) The change appears to be due at least in part to the direct effect of heat on the cells, it is usually accompanied by actual fragmentation leading to the production of extremely small microspherocytes Spherocytosis is also characteristically found in the acute hæmolytic anæmia associated with *Cl welchii* septicæmia

"**Irregularly Contracted Erythrocytes** Shrunken and distorted erythrocytes are not uncommonly seen in peripheral blood films in certain types of hæmolytic anæmia Following ingestion of hæmolytic poisons such as acetylphenylhydrazine contracted corpuscles of irregular outline are conspicuous (Fig 5) Similarly distorted corpuscles were observed by Brookfield (1928) in acute lead poisoning by Stats Wasserman and Rosenfield (1948) in hæmolytic anæmia due to sulphapyridine and by Zuelzer and Apt (1949) in anæmia due to naphthalene (moth ball) poisoning The cell distortion presumably results from the direct action of the poison upon the erythrocyte An increase in osmotic fragility indicates that the cell surface is damaged This type of erythrocyte distortion, although typical of that produced by some hæmolytic poisons may rarely be observed in hæmolytic anæmia of endogenous origin *e g* in the hæmolytic anæmias associated with methæmoglobin and/or sulphæmoglobin formation (Fig 6)

Two other probably distinct types of deformed and contracted corpuscles have been described One is the 'burr' cell which Schwartz and Motto (1949) observed in small numbers in various blood disorders and in large numbers in uræmia carcinoma of the stomach and bleeding peptic ulcer The other is the 'triangular cell' of Dacie and co workers (1953) which was found in large numbers in the blood of a young girl suffering from a chronic hæmolytic anæmia associated with uræmia and thrombocytopenia (Fig 8) (One or other type of cell or both have since been reported in acute progressive uræmia acute hepatic necrosis and carcinomatosis by Dacie (1954) and in acute uræmia accompanied by hæmolysis by Gasser and his colleagues (1955) Allison (1957) and Aherne (1957) They also occur in thrombotic thrombocytopenic purpura)

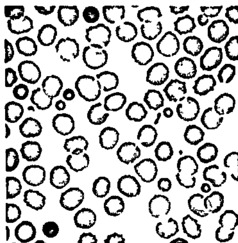


Fig 4 Photomicrograph of a bl od film of a patient wh had been severely burned Th very small sp erical cell are fragments of erythrocytes d rectly affected by heat × 00

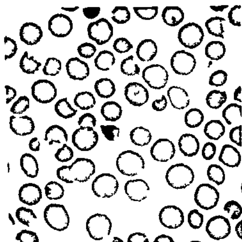


Fig 5 Photom crograph of a blood f lm of a patient with poly cythæm a vera wh o had receiv d an o r dose of acetylphenyl hydr n The irregularly ntr cted da kly staining corpu cles cont a t m k dly with th s affected mo l ghtly stainin cell × 700

T f p –



Fig. 6. Photomicrograph of a blood film of a patient suffering from a hæmolytic anæmia of obscure origin associated with methæmoglobinæmia and sulphæmoglobinæmia. Many irregularly contracted corpuscles are present. × 700

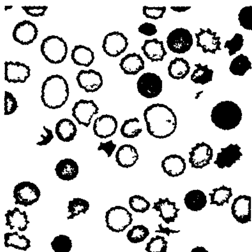


Fig. 7. Photomicrograph of a blood film of a patient suffering from carcinomatosis and hæmolytic anæmia (Case 2 of Dacie 1954). Many irregularly crenated and contracted corpuscles are present (? burr cells). × 700

The author believes that burr cells and triangular cells are distinct entities though both types of cell may be present at the same time. Burr cells or irregularly crenated cells appear to be artefacts produced on the slide as the cell dries; they are difficult to see in wet preparations of blood. Fig. 7 illustrates typical burr cells and shows stages in their formation. Triangular cells as illustrated in Fig. 8 have smoother outlines; they rarely have more than two spines and may have none. Some are conspicuously triangular in shape; they are always microcytes and stain deeply with Romanowsky dyes. Some at least of the cells have an increased osmotic fragility. They may be readily seen in wet preparations of blood and presumably circulate as such in the blood stream. Reticulocytes are not affected.

Burr cells thus seem to owe their origin to irregular crenation *in vitro* and triangular cells to fragmentation of adult cells *in vivo*. Burr cells are found in a variety of conditions including uræmia, and small numbers can often be seen in blood films of children suffering from a variety of infective diseases. They can also be found in blood films from patients who have undergone splenectomy for any cause. It is doubtful whether their presence denotes increased hæmolysis. Triangular cells are probably always associated with increased hæmolysis and may be looked upon as prehæmolytic forms. As mentioned above they are found in acute progressive uræmia in adults, in a hæmolytic uræmic syndrome in children and in thrombotic thrombocytopenic purpura.

The exact pathogenesis of both burr cells and triangular cells is unknown.

Schistocytes

The products of erythrocyte fragmentation were referred to by Ehrlich (1891) as "Schistocyten" and by Rous and Robertson (1917) as "schizocytes". Such cell fragments are seldom seen in preparations of normal human blood. When visible in appreciable numbers their presence is good evidence of a hæmolytic process (Fig. 9). Normally it seems likely that any fragments formed are quickly sieved out of the circulation by the spleen (see Robertson and Rous, 1917).

As previously mentioned, severe burns cause fragmentation as well as spherocytosis, and similar changes result from heating blood *in vitro* (Shen, Ham and Fleming, 1943; Brown, 1946; Ham, Shen, Fleming and Castle, 1948). In human patients the fragments resulting from severe burns disappear from the circulation within a few hours.

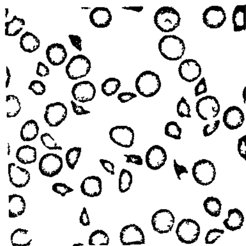
The triangular cells referred to above may also be looked upon as a special type of schistocyte. Fragmentation is a conspicuous feature of certain types of congenital hæmolytic anæmia, *e.g.* hereditary elliptocytosis (Fig. 9) and Mediterranean anæmia (Fig. 85, p. 208). Occasionally in cases of hæmolytic anæmia a few of the erythrocytes appear as if part of their substance had been

indented and pulled outwards by means of a pair of pincers (Fig 10) Dacie and co workers (1953) observed these ' pincered cells in quite large numbers in a patient suffering from a hereditary non spherocytic hæmolytic anæmia they were found in smaller numbers in other types of hæmolytic anæmia Presumably they too represent corpuscles in the process of fragmentation Rous and Robertson (1917) observed similar cells in normal rabbit blood and larger numbers in blood from rabbit spleen

Erythrophagocytosis

Human erythrocytes which have undergone phagocytosis by monocytes or neutrophils are rarely found in blood films Never theless they have been seen from time to time in small numbers in many types of hæmolytic disorder such as those associated with chemical poisoning septicæmia and protozoal infections and in hæmolytic disease of the newborn paroxysmal cold hæmoglobinuria and acquired hæmolytic anæmia of both the warm and cold auto antibody types (Fig 11) Zinkham and Diamond (1952) showed that in acquired hæmolytic anæmia the number of phagocytes containing erythrocytes might be greatly increased if the patient s blood was incubated *in vitro* at 37° C for 30 to 120 minutes before smears were made It seems possible that in man at least phagocytes containing erythrocytes are rapidly removed from the circulation perhaps particularly by the spleen and lungs (In animals such as the rat however erythrophagocytosis in the peripheral blood is a marked feature of experimentally induced hæmolytic anæmia) (Bessis and Freixa 1947) Bonnin and Schwartz (1954) made a detailed study of the ability of different types of antibodies to cause erythrophagocytosis *in vitro* It was found that only those antibodies which were capable of causing hæmolysis in the presence of complement regularly caused erythrophagocytosis Monocytes appeared to be more active as erythrophages than neutrophils for the latter only enveloped corpuscles which had been sensitized by high concentrations of antibody

- Ehrlich (1891) seems to have been the first to observe erythropha gocytosis in man He placed a ligature around the finger of a patient with paroxysmal cold hæmoglobinuria chilled the finger and subsequently found erythrophagocytosis in blood taken from the chilled finger Schubothe and Müller (1955) have recently resuscitated Ehrlich s test by applying it to a variety of hæmolytic disorders and exposing the finger to a range of temperatures In paroxysmal cold hæmoglobinuria 10 minutes at 5 C followed by 10 minutes at 40 C gave rise to marked hæmolysis and erythrophagocytosis in the



I tc 8 I hotomierograpl cfa I loc l film of a pati nt sffering from a ( ) con renital h molyti anreia vith tl rombocytopenia (hronic nej l riti lc l t) l ath from uræmia (Case 1- cf Dacie *et al* 19o3) Many triangul r corpuscl are present Note al sence cf crenati n (*f* Fig ") × 00

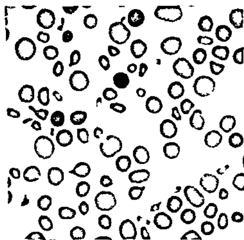


FIG 9 I hotomicogra l of a l lood film of a chil l suffering from a congenital læmolyti anæmia (probal ly hered tary ell ptocytosi see p 160) (Ca e 11 of Dacie *et al* 19o3) Numerous cell fra ments (schistocytes) can l e seen as well as microspherocytes × "00

T fa p 24

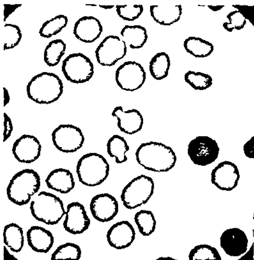


Fig 10 *Photomicrograph of a blood film of a man suffering* from hæmolytic anæmia of unknown type associated with hæmoglobinuria Several pincered cells undergoing fragmentation can be seen See also Fig 78 × 1000



Fig 11 Photomicrograph of a blood film of a patient suffering from idiopathic acquired hæmolytic anæmia (warm antibody type) Two monocytes can be seen one of which has phagocytosed an erythrocyte (*marked with an arrow*) (Case 1 of Dacie 1954) × 700

cold hæmagglutinin syndrome 10–30 minutes at 20° C led to marked hæmolysis and a little erythrophagocytosis in acquired hæmolytic anæmia of the warm auto-antibody type 10–30 minutes at 40° C gave rise to a moderate amount of erythrophagocytosis but no hæmolysis In paroxysmal nocturnal hæmoglobinuria 10–30 minutes at 40° C, led to marked hæmolysis but doubtful erythrophagocytosis The results which may be obtained using the Ehrlich test thus reproduce closely what may be observed experimentally *in vitro*

Heinz Bodies

Riess in 1882 noticed unusual rounded globules and granules in the erythrocytes in potassium chlorate poisoning Similar intracorpuscular bodies were later described in greater detail by Heinz (1890) in the blood of guinea pigs poisoned with pyrodin (acetylphenylhydrazine) Heinz bodies are now known to be produced by the action on the blood of a wide range of aromatic nitro and amino compounds as well as by inorganic oxidizing agents such as potassium chlorate Heinz bodies may be found in the absence of anæmia but large doses of all the drugs that cause Heinz body formation cause hæmolysis Recent reviews on Heinz bodies include those of Webster (1949) Buckell and Richardson (1950) and Fertman and Fertman (1955)

Heinz bodies probably consist of denatured globin derived from hæmoglobin The chemical changes leading to their formation are complicated and have been the subject of much controversy According to Brenner and Allison (1953) inhibition of catalase may be a first step or one of the probable steps in the production of Heinz bodies In the case of acetylphenylhydrazine Beaven and White (1954) suggested that hæmoglobin acts as a catalyst in the oxidation of the acetylphenylhydrazine and is itself broken down in the process Methæmoglobin is not usually formed under conditions which favour Heinz body formation

A recent noteworthy discovery has been that certain human erythrocytes produce Heinz bodies as a result of chemical action unusually readily both *in vivo* and *in vitro* This undue sensitivity appears to be the basis of the hæmolytic anæmia which occurs in a small percentage of negro subjects given the antimalarial drug primaquine (Beutler Dern and Alving 1955) (see Chapter 15) The mechanism of the sensitivity is thought to be a lowered glucose 6 phosphate dehydrogenase activity (Carson *et al* 1956) A similar or comparable metabolic defect may be the cause of the relatively frequent occurrence of Heinz bodies in premature infants

Morphologically Heinz bodies are refractile rounded bodies often with a slightly irregular contour ranging in size from

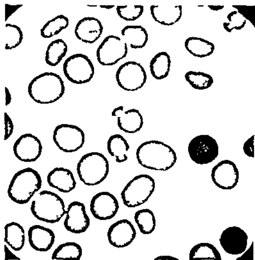


Fig 10 Photomicrograph of a blood film of a man suffering from hæmolytic anæmia of unknown type associated with hæmoglobinuria Several "puckered" cells undergoing fragmentation can be seen See also Fig 78 × 1000



Fig 11 Photomicrograph of a blood film of a patient suffering from idiopathic acquired hæmolytic anæmia (warm antibody type) Two monocytes can be seen one of which has phagocytosed an erythrocyte (*marked with an arrow*) (Case 1[illegible] of Dacie 1954) × 700



Fig 1 Photomicrograph of a blood film of a patient suff ring from acetylphenylhydrazine poisoning (after splenectomy) (Case 31 of Dacie 19 4) Nearly every corpuscle contains a large Heinz body Stained supravitally by methyl violet ×700

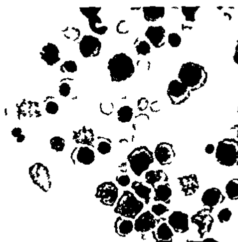


Fig 13 Photomicrograph of a film of sternal bone marrow from a patient suffering from paroxysmal nocturnal haemoglobinuria (Case 3. of Dacie 1954) Normoblasts in all stages of development are the predominating cell × 400

T f ce p 8.

minute particles to bodies up to 3 μ in size (Fig 12) Several small bodies may be present in the same cell the largest ones are usually present singly They are easily visible in unstained or wet preparations of blood they stain supravitally with a range of basic dyes including methyl violet used by Heinz himself and brilliant cresyl blue However they are not usually discernible in Romanowsky stained preparations or in brilliant cresyl blue stained films fixed in methanol before counterstaining They are generally only seen in fully ripened corpuscles and not in reticulocytes (but see Dacie (1954 p 401)) In cresyl blue stained preparations they stain a distinctly lighter shade of blue than the reticular filamentous material of reticulocytes

Heinz bodies occur in the blood in larger numbers after splenectomy (Webster 1949 see also p 196) It is possible that corpuscles containing Heinz bodies are selectively retained by the spleen or even that the spleen removes Heinz bodies from intact erythrocytes Congenital Heinz body anæmia in man is considered in Chapter 4 (p 195) and that produced by drugs in Chapter 15

COMPENSATORY ERYTHROPOIESIS IN HÆMOLYTIC ANÆMIA

In hæmolytic anæmia the output of new erythrocytes from the bone marrow usually increases in step with the increased rate of hæmolysis In this way some measure of compensation for the hæmolysis is generally achieved In most instances when the hæmolytic process is a chronic one a fairly steady balance between destruction and formation is established at an erythrocyte level below the normal Occasionally compensation is complete and the patient manages to maintain a normal erythrocyte count and hæmoglobin level In severe hæmolytic states adequate compensation may be impossible with the result that the patients rapidly become seriously anæmic

There is of course a limit to the numbers of erythrocytes that can be produced and the amount of hæmoglobin that can be synthesized daily Crosby and Akeroyd (1952) calculated from knowledge of the total circulating hæmoglobin and mean erythrocyte life span in cases of hæmolytic anæmia that as much as 6–8 times the normal amount of hæmoglobin might be produced daily but no more This is equivalent to as much as 0 6 g of hæmoglobin per kg of body weight daily e g 42 g in a 70 kg man

The amount of hæmoglobin synthesized daily by a healthy adult can be calculated as follows



Fig. 12. Photomicrograph of a blood film of a patient suffering from acetylphenylhydrazine poisoning (after splenectomy) (Case 31 of Dacie, 1954). Nearly every corpuscle contains a large Heinz body. Stained supravitally by methyl violet. × 900

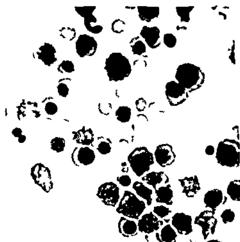


Fig. 13. Photomicrograph of a film of sternal bone-marrow from a patient suffering from paroxysmal nocturnal haemoglobinuria (Case 32 of Dacie, 1954). Normoblasts in all stages of development are the predominating cells. × 400

To face p. 6.

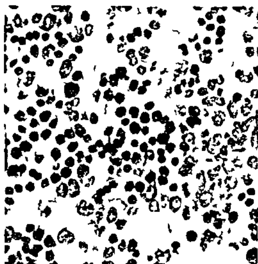


Fig 14 Photomicrograph of a section of sternal bone marrow aspirated from a patient suffering from hereditary spherocytosis The marrow is hyperplastic and normoblasts are conspicuous H and E × 460

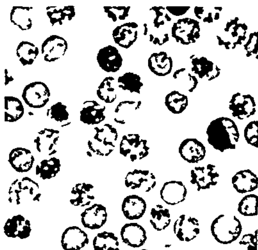


Fig 15 Photomicrograph of a blood film of a patient suffering from hereditary non spherocytic hæmolytic anæmia (Type II (Case 1 of Dacie et al 1953) Almost all the cells are reticulocytes Stained supravitally with brilliant cresyl blue × 1000

The total erythrocyte volume in health is approximately 30 ml per kg of body weight If the normal MCHC is 33 this is equivalent to $\frac{30 \times 33}{100} = 9.9$ g of hæmoglobin per kg If it is assumed that 1/120th of the circulating hæmoglobin is catabolized and renewed daily the normal daily *production of hæmoglobin* is then $\frac{9.9}{120} = 0.0825$ g per kg In a 70 kg man this is equivalent to approximately 5.8 g daily As mentioned above the maximum synthesis in hæmolytic anæmia may reach 6–8 times this amount *i e* 35–45 g daily (For a discussion of failure of bone marrow compensation see p 20)

Increase in erythropoiesis is brought about by hyperplasia of the bone marrow The erythroid myeloid ratio in the marrow rises from the normal average proportion of about 1 in 5 to 1 in 1 in extreme instances erythropoietic cells may actually predominate (Fig 13) The fat cells in the marrow tend to disappear so that the marrow may become solidly cellular (Fig 14) The volume of active marrow increases and red marrow develops in the long bones and in other sites in adults where it does not normally occur Occasionally centres of extramedullary formation are found (see p 114)

In children the possibilities of hyperplasia of the marrow are more limited as nearly all the medullary cavities are normally *occupied by active hæmopoietic marrow If the stimulus for* increased erythropoiesis is sufficiently great hyperplasia may then result in an actual increase in the size of the medullary cavities In the skull this may lead to an obvious widening of the diploe *Extramedullary foci are possibly more frequent in children for* the same reason

The hyperplasia of erythropoietic marrow is reflected in certain definite changes in the peripheral blood picture These changes are *reticulocytosis macrocytosis* and *erythroblastæmia*

Reticulocytosis in Hæmolytic Anæmia The normal range for the reticulocyte count is usually given as between 0.2% and 2.0% in adults In hæmolytic anæmia the proportion is often as high as 20% occasionally the count is much higher and may reach 70% or even more (Fig 15) Although there is no very close correlation between reticulocyte counts and the degree of anæmia the highest counts are generally found in the more anæmic patients in whom hæmolysis is usually more intense and the efforts at compensation consequently greater

Used as an assessment of marrow activity the reticulocyte count should be related to the degree of anæmia present at the time either by

expressing the count in absolute numbers normally about 25 000–100 000 reticulocytes per cu mm or by multiplying the observed percentage by the ratio between the patient s packed cell volume or hæmoglobin level to the normal 45% or 15 g (Giblett *et al* 1956) Sometimes in well compensated hæmolytic anæmia the reticulocyte count seems to be disproportionately high (*e g* Fig 15) this probably means that the erythrocytes are leaving the bone marrow at an unusually early stage in maturation as shown by their heavy reticulum and have an abnormally long maturation time in the peripheral blood Examples of very different reticulocyte counts in two patients suffering from different types of congenital non spherocytic hæmolytic anæmia who had almost identical erythrocyte life spans are quoted in Chapter 4 (p 190) In untreated pernicious anæmia there is reason to believe on the other hand that the erythrocytes remain in the marrow for an unusually long time and lose or almost lose their reticulum before entering the peripheral blood (Finch *et al* 1956) The peripheral blood reticulocyte count in such cases thus gives an underestimate of marrow activity

Hollingsworth (1955b) also made the point that the reticulocyte count is an unreliable index of erythropoietic activity In studies on the rate of erythrocyte glycolysis in various hæmolytic disorders he found that although the rate of glycolysis was usually raised in association with raised reticulocyte counts there were some exceptions in which the reticulocyte counts were unexpectedly low although the rate of glycolysis was high This was particularly noticeable in homozygous Hb C disease

Failure of Regeneration Occasionally the erythropoietic activity of the bone marrow fails in the course of a chronic hæmolytic anæmia with the result that the reticulocyte count falls to very low levels A serious increase in anæmia may result Although best known in hereditary spherocytosis (Owren 1948) aplastic crises are now known to occur in other hæmolytic anæmias also *e g* in sickle cell disease acquired hæmolytic anæmia paroxysmal nocturnal hæmoglobinuria and hæmolytic disease of the newborn

The changes in marrow erythroblasts in acute crises have been variously described possibly due to the different times in the course of the crises at which the samples were obtained Owren (1948) reported the virtual disappearance of normoblasts early in the crisis with the marrow then consisting almost exclusively of leucopoietic tissue After a few days regeneration started primitive cells with basophilic cytoplasm first appeared and then successively proerythroblasts (macroblasts) and normoblasts Owren (his Fig 7) illustrated a binucleated macroblast and the prevalence of giant proerythroblasts sometimes multinucleated when regeneration first starts has subsequently been mentioned by Undritz (1949) and Gasser (1957) Dameshek and Bloom (1948) finding at a time when no reticulocytes were present in the peripheral blood a hyperplastic bone marrow containing many pronormoblasts but no mature normoblasts concluded that the basis of the crisis in the

patient they studied was an arrest in the maturation of the erythropoietic tissue (see also p 112)

The reasons for acute (transitory) or chronic marrow failure are poorly understood Toxic or infective processes appear to be the main causes of acute marrow hypoplasia but (in acquired hæmolytic anæmia damage to the nucleated red cells by the auto antibodies may be the cause) The failure of erythropoiesis cannot as a rule be satisfactorily accounted for by deficiencies of known hæmopoietic materials However recent studies have shown that deficiency of folic acid is not uncommon in cases of hæmolytic anæmia (or leukæmia and allied disorders) and that occasionally the deficiency is sufficiently great to cause overt megaloblastic change (Chanarin Dacie and Mollin 1959) In such cases giant metamyelocytes are conspicuous in bone marrow films and well developed megaloblasts may also be present (Fig 17) However although the reticulocyte count in the peripheral blood may be relatively low the marrow is hyperplastic and megaloblastic not aplastic

As mentioned on p 20 the whole question of the erythropoietic response to hæmolysis was considered in detail by Crosby and Akeroyd (1952) They pointed out that if the maximum possible output of hæmoglobin by a healthy adult was about 0 6 g per kg per day *i e* about 6–8 times the normal then in theory at least hæmolysis can occur at about six times the normal rate corresponding with a mean erythrocyte life span as low as 20 days without the patient necessarily becoming anæmic Crosby and Akeroyd calculated the probable hæmoglobin output of patients suffering from Mediterranean anæmia and pernicious anæmia respectively and found that this was far less than 0 6 g per kg per day

The same problem was considered by Finch and Coleman (1953) They studied the degree of erythropoietic hyperplasia in the marrow the rate of appearance of ^{59}Fe in the hæmoglobin of the peripheral blood and the morphology of the patients erythrocytes They concluded that three types of erythropoiesis could be differentiated compensated decompensated and dyservthro poiesis In the compensated type the mass of erythropoietic tissue was increased but maturation took place normally and the erythrocyte morphology was normal except for the effects of the hæmolytic process In the decompensated type the mass of erythropoietic tissue in the marrow was increased but the maturation of erythroblasts was accelerated and poikilocytes siderocytes and abnormally large numbers of reticulocytes were present in the peripheral blood In patients showing

dyserythropoiesis the production of erythrocytes from the marrow fell far short of the marrow s potential capacity

Further studies on erythrokinetics employing ^{59}Fe and in some instances ^{51}Cr *have recently been published Giblett and her co workers* (1956) carried out an elaborate study on nineteen normal subjects and twenty five anæmic patients Their measurements included bone marrow cellularity (erythroid myeloid ratio) reticulocyte count fæcal urobilinogen (expressed as the absolute daily excretion and as mg per g hæmoglobin per day) plasma half clearance time of ^{59}Fe plasma *iron turnover and erythrocyte utilization of radioactive iron On the* basis of their data they were able to distinguish four categories of marrow function (*a*) hyperfunction (*b*) relative hypofunction (*c*) failure and (*d*) dysfunction Seven cases of hæmolytic anæmia were studied all fell in the marrow hyperfunction categories patients with pernicious anæmia or Cooley s anæmia fell in the marrow dysfunction group

They also distinguished between total erythropoiesis and effective erythropoiesis the plasma iron turnover fæcal urobilinogen excretion and erythroid myeloid ratio were taken to be indicators of total erythropoiesis and the reticulocyte count red cell utilization of radio active iron and erythrocyte life span measurement reflections of effective erythropoiesis

Effective erythropoietic activity *thus represents the production* of erythrocytes which have a measurable life span in the peripheral blood while the total activity includes effective activity plus erythroblast proliferation and/or hæm synthesis not resulting in viable erythrocytes The data of Giblett and her co workers in hæmolytic anæmia indicate that erythrocyte production may be increased 4–8 times *Giblett and her co workers pointed out that the maximal* rate of erythropoiesis is found in congenital cases of hæmolytic anæmia which suggests that time may be an important factor in achieving compensation In anæmia of short duration as in acute blood loss blood production may only reach 2–3 times the normal even in the presence of adequate iron stores while in secondary anæmias such *as those in association with cirrhosis leukæmia uræmia and arthritis* marrow dysfunction is usually at least as important as hæmolysis in the pathogenesis of the patient s anæmia

Bothwell Callender Mallett and Witts (1956) have also reported extensive data on erythropoiesis based on studies carried out with ^{59}Fe Eight patients with hæmolytic anæmia were included in their *series The plasma iron turnover was increased in all of them but the* erythrocyte utilization of ^{59}Fe was variable

Bothwell Hurtado Donohue and Finch (1957) using ^{59}Fe have published further extensive data The subjects studied included fourteen normal subjects sixty seven patients with blood disorders and four with hæmochromatosis They concluded (*a*) that the plasma iron turnover is not affected by the rate of erythrocyte destruction and is only affected to a limited extent by the body stores of iron (*b*) that it reflects the degree of erythropoietic activity being increased in marrow hyperfunction to 3–6 times the normal and (*c*) that it is increased too in marrow dyshæmopoiesis where it is a measure of total erythropoiesis rather than a measure of the production of viable cells

Studies with radioactive iron thus provide useful information on erythropoiesis and in cases of hæmolytic anæmia they may help to explain a degree of anæmia not satisfactorily explained solely on the degree of hæmolysis The ability to carry out combined and not successive studies using ^{59}Fe and ^{51}Cr is an advantage Technical details of methods are given by Weinstein and Beutler (1955) Joske McAlister and Prankerd (1956) and Mitchell Spencer and King (1957) The whole concept of relative bone marrow failure is succinctly reviewed by Moore (1957)

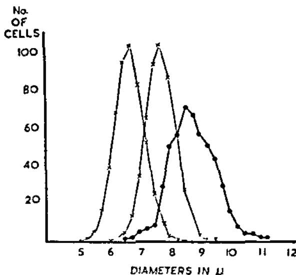


FIG 10 Erythrocyte diameter distribution curve (Price Jones curve) made from a dried peripheral blood film of a patient suffering from hereditary non spherocytic hæmolytic anæmia (Type II) (Case 1 of Dacie *et al* 1953) MCD = 8 7 μ S D = 0 77 μ. The thin outlines indicate the maximum and minimum normal curves

Macrocytosis An increase in the average size of the erythrocytes in the peripheral blood seems to be a regular accompaniment of increased erythropoiesis whether this is a response to hæmorrhage (Lehmann 1949 Wintrobe 1951) or to hæmolysis (Dameshek and Schwartz 1940) The cause of the macrocytosis is uncertain the cells are presumably derived from unusually large precursors macronormoblasts (Dacie and White 1949) The macrocytosis is generally accompanied by an increased proportion of reticulocytes in the peripheral blood but the high proportion of reticulocytes present cannot be the whole explanation

for the macrocytosis for the fully ripened corpuscles are also mostly larger than normal This increase in size is reflected in an increase in mean cell diameter as well as in an increase in mean cell volume (Fig 16) Observations on the mean cell volume of a

Table 1

Mean Corpuscular Volume (MCV) Mean Corpuscular Hæmoglobin Concentration (MCHC) and Maximum Reticulocyte Counts in Various Types of Congenital and Acquired Hæmolytic Anæmia

Type of hæmolytic anæmia	MCV (cu μ)		MCHC (%)		Reticulocytes (maximum count) (%)	
	Range	Mean	Range	Mean	Range	Mean
Hereditary spherocytosis	70–105 (42)	84	32–40 (32)	36 4	3–41 (48)	15 1
Hereditary non spherocytic hæmolytic anæmia						
Type I (13)	76–119	100	28–34	31 2	3 [illegible]–23	11 8
Type II (2)*	111–150	132	27–34	30 8	40–75	56
Idiopathic acquired hæmolytic anæmia						
Warm antibody type (12)	88–130	111	[illegible]7 5–39	3[illegible] 7	8–57	28 4
Cold antibody type (6)	90–129	110	26–38	33 0	1–16	10 7
Paroxysmal nocturnal hæmoglobinuria (12)	89–145	118	28–33	30 5	1–55	[illegible]2 4
Normal range	76–96	86	32–36	34	0 2–2 0	

The number of patients studied is indicated by the number in parentheses
* After splenectomy

series of patients with hæmolytic anæmia are given in Table 1 Where there is conspicuous spherocytosis the contrast between the macrocytic reticulocytes and spherocytic fully ripened corpuscles is often most striking

Erythroblastæmia Normoblasts are not infrequently present

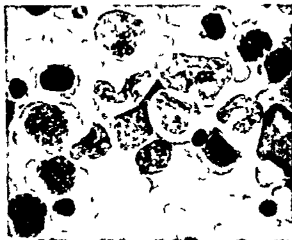


FIG 37 Photomicrograph of a film of sternal marrow of a patient suffering from idiopathic acquired hæmolytic anæmia (warm antibody type). Two intermediate megaloblasts can be seen (at left of centre) and one giant metamyelocyte (at right of centre). × 900. (From Chanarin, Dacie and Mollin (1959).)

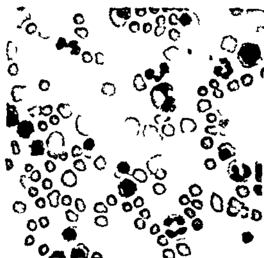


Fig. 18 Photomicrograph of a blood film of a patient suffering from idiopathic acquired hæmolytic anæmia (warm antibody type). Hæmolysis persisted after splenectomy. Numerous normoblasts and a single erythrophage are present. × 400

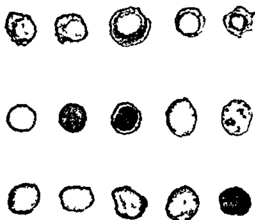


Fig. 19 Photomicrograph of normoblasts and erythrocytes stained by Perls's reaction to show siderotic granules (top two rows) and stained by Jenner-Giemsa stain to demonstrate Pappenheimer bodies (bottom row). (From Douglas and Dacie 1953.) × 1000

in the peripheral blood of patients with hæmolytic anæmia Usually however there are less than 1 per 100 leucocytes In general the higher the reticulocyte count and the more anæmic the patient the more frequent are the normoblasts In young children however erythroblastæmia may be a well marked feature in many types of hæmolytic anæmia particularly is this so in hæmolytic disease of the newborn In adults the presence of large numbers of normoblasts should cause the observer to reconsider the diagnosis of a primary hæmolytic anæmia However it is not uncommon to find numerous normoblasts in the peripheral blood of patients suffering from acquired hæmolytic anæmia of the auto-antibody type in whom hæmolysis has persisted at a rapid rate following splenectomy (Fig 18)

Siderocytosis

Siderocytes are erythrocytes containing granules giving Perls s Prussian blue reaction for ionized ferric iron They were observed by Gruneberg (1941a b) in small numbers in normal rat mouse and human embryos and in large numbers in mice with a congenital anæmia (Gruneberg 1942) They were first recognized in adult human blood by Doniach Gruneberg and Pearson (1943) It is now realized that although siderocytes are not present in the peripheral blood of children or adults in health except possibly in extremely small numbers (<0 01%) they are present in the cord blood of newborn infants and in the peripheral blood in a wide range of blood disorders particularly after splenectomy (Douglas and Dacie 1953) Siderotic granules at any rate those demonstrated by the simple HCl potassium ferrocyanide method do not indicate a hæmolytic process nor does their presence indicate a dying cell (*cf* Case 1945) on the contrary the granules appear in developing erythroblasts at the same time as hæmoglobin is being formed (Gruneberg 1942 Dacie and Doniach 1947) The siderotic granules stain also with Romanowsky dyes and when stained in this way were referred to by McFadzean and Davis (1947) as Pappenheimer bodies (Pappenheimer Thompson Parker and Smith 1945) (Fig 19)

The incidence and significance of siderocytes were briefly touched upon by Dacie and White (1949) in a review on erythropoiesis in general they suggested that (a proportion of the iron which normally enters the hæm molecule is concentrated in loci in the cytoplasm of the developing normoblast in a relatively free form ") Douglas and Dacie (1953) later published data based on the study of the peripheral blood and bone marrows of a relatively large

series of normal subjects and patients with a variety of blood disorders including hæmolytic anæmia Their findings may be summarized as follows a few small iron containing granules may be recognized in a large proportion of the normoblasts of normal bone marrow a small number of normal marrow reticulocytes are siderocytes but very few if any of the reticulocytes of the peripheral blood The proportion of normoblasts containing iron granules is increased and the granules may be unusually numerous and large in diseases where there is a defect in hæmoglobin synthesis or erythropoiesis In iron deficiency on the other hand iron containing granules are absent from the normoblasts The presence of iron containing granules in normoblasts is thus a normal phenomenon It appears that more iron is taken into the erythroblast during hæmoglobin synthesis than can be immediately incorporated into hæm and that this excess iron may be utilized during the later stages of normoblast ripening and during the *early reticulocyte stage*

Siderocytes are seldom seen in the peripheral blood in patients with blood diseases except after splenectomy when they may often be found in very large numbers Splenectomy does not appear to cause any increase in the numbers of erythroblasts containing iron granules in the marrow nor could Douglas and Dacie demonstrate that the spleen filtered off siderocytes from the circulation as has been claimed (McFadzean and Davis 1949 Pirrie 1952) Douglas and Dacie found the highest siderocyte counts in the peripheral blood of patients with high reticulocyte counts which had persisted after splenectomy or of patients suffering from defects in hæmoglobin synthesis who had undergone splenectomy (Fig 95 p 228)

Crosby (1953) suggested that the spleen in some way removes siderotic granules from erythrocytes without actually destroying the cells He transfused blood containing many siderocytes obtained from a patient who had undergone splenectomy into a recipient who had a normal spleen and found that the siderotic granules disappeared within 3 hours although the transfused cells were not destroyed In a later paper Crosby (1957b) described six experiments In four of them blood containing many siderocytes was transfused to recipients with intact spleens within 4 hours more than half the siderocytes had disappeared although the transfused cells had not apparently been destroyed to any appreciable degree Two other recipients had undergone splenectomy 80% of the siderocytes and 90% of the transfused cells were still present 24 hours later

Crosby's experiments indicate without doubt that the spleen has the remarkable property of removing siderotic granules from erythrocytes without actually destroying the cells. Whether the *spleen removes the granules intact* as suggested by Crosby, or in some way accelerates their metabolism within the cells, as suggested by Douglas and Dacie (1953), remains to be seen.

More recent papers on the siderocyte problem include those of Kaplan, Zuelzer and Mouriquand (1954), Morse (1955), Bessis and Breton-Gorius (1956, 1957) and Mouriquand (1958).

Kaplan, Zuelzer and Mouriquand (1954) studied 155 infants and children, including hæmatologically normal subjects. Bone marrow was aspirated from the iliac crest or tibia. They confirmed that normoblasts containing stainable granules of iron (which they termed "sideroblasts") were demonstrable in the bone marrow in health and that they were only absent in iron-deficiency states associated with hypoferræmia. Twenty-seven of their patients had hæmolytic anæmia of one type or another. The percentage of sideroblasts tended to be higher than normal and in some, but not in all, the size and staining intensity of the granules appeared to be slightly greater than normal.

Morse (1955) studied the bone marrows and peripheral blood of (? adult) patients suffering from a variety of chronic diseases and blood disorders, and correlated the proportion of normoblasts in the marrow containing siderotic granules with the stainable marrow iron and the *serum iron level*. *He also confirmed that the proportion of iron*-containing normoblasts was low in hypoferræmia and found unusually high numbers in dyshæmopoietic anæmias such as pernicious anæmia, thalassæmia and erythræmic myelosis.

Bessis and Breton-Gorius (1956, 1957) studied the siderotic granules of erythrocytes and normoblasts by electron microscopy. They found that they consist of collections of very fine dense granules, 40–100 Å in diameter. These granules disperse throughout the cell in the course of hæmoglobin synthesis. They appear to be absorbed as such into the erythroblast cytoplasm from adjacent reticulum cells. In the hæmoglobinopathies and in experimental lead poisoning enormous numbers of the minute granules were visible in masses or as a diffuse dust.

Mouriquand (1958) in a recent review discusses the morphology and significance of the sideroblast and summarizes the literature.

SPECIAL LABORATORY TESTS USEFUL IN INVESTIGATING THE HÆMOLYTIC ANÆMIAS

The tests to be described in this section are the osmotic and mechanical fragility tests, and certain serological procedures. They will be discussed in general terms only, with particular

reference to their significance in diagnosis Further details will be given later when the various types of hæmolytic anæmia are described Technical details are given by Dacie (1956)

Osmotic Fragility

The introduction of the fragility test into clinical laboratory practice seems to have quickly followed the pioneer observations of Chauffard (1907) on the decreased resistance to hypotonic salt solution of the erythrocytes in l ictère congenital de l adulte (hereditary spherocytosis) Although the correlation between a reduction in the diameters of mammalian erythrocytes and increase in osmotic fragility had been demonstrated by Vallery Radot and Lhéritier in 1919 it was not until Haden s (1934) paper that increased osmotic fragility was satisfactorily correlated with spherocytosis Other confirmatory publications followed (Castle and Daland 1937 Dacie and Vaughan 1938) and it is generally held to day that erythrocyte shape is a major factor in determining osmotic fragility In Sharpsteen s (1955) view however it is an increase in intracellular protein osmotic pressure rather than cell shape that is responsible for the raised osmotic fragility in hereditary spherocytosis

It is quite clear that increased osmotic fragility is not the monopoly of any particular type of hæmolytic anæmia Definite increases in fragility are found in hereditary spherocytosis (almost invariably) in auto immune hæmolytic anæmia (most patients) in hæmolytic disease of the newborn due to anti A (less commonly in sensitization due to anti Rh) in hæmolytic anæmia due to chemical poisoning and in severe burning etc *i e* in just those cases in which spherocytosis is usually obvious in blood films However it should be added that sometimes deviations from the normal are slight and that a carefully standardized technique is required to detect them Osmotic fragility is almost normal or normal in most cases of secondary or symptomatic hæmolytic anæmia and in paroxysmal nocturnal hæmoglobinuria In Mediterranean anæmia sickle cell anæmia and in other allied disorders there is characteristically an increased resistance to hæmolysis with or without a small proportion of unusually fragile cells

A number of variants of the osmotic fragility test have been introduced from time to time Some of the variations are technical ones and are concerned with such things as the way in which hæmolysis is measured the proportion of blood added to saline and whether the tonicity of the blood saline suspension is corrected

for the amount of blood added and for alteration in pH (Emerson et al 1956) Other variations have involved the use of unusual hæmolysing solutions for instance Dickstein and co workers (1949) employed in addition to simple hypotonic saline solutions of glycerine and thiourea in hypotonic saline The important thing is for the test to be carried out in as completely a standardized way as possible The author has used a slight modification of the method of Parpart and co-workers (1947) The range of results in health based on a study of 30 adult men and 22 adult women is given below (Table 2)

Table 2

*Normal Range of Erythrocyte Osmotic Fragility**

% NaCl	% Lysis
0.30	97–100
0.35	90–99
0.40	50–95
0.45	5–45
0.50	0–6
0.55	0
Median corpuscular fragility (MCF) = 0.40–0.445% NaCl	

* Based on observations made on 30 healthy adult men and 22 healthy adult women

The tests were carried out at 20° C, and at pH 7.4

Recording the Results of Osmotic Fragility Tests Most workers have not been content to record merely the highest concentration of saline at which hæmolysis is just detectable (initial lysis or minimum resistance) and the highest concentration of saline in which hæmolysis appears to be complete (complete lysis or maximum resistance) It is advantageous at least to record in addition the concentration of saline causing 50% lysis (median corpuscular fragility (MCF) Vaughan 1937 Dacie and Vaughan 1938) It is worth while too when a range of hypotonic solutions has been used to construct a fragility curve by plotting on graph paper the percentage of hæmolysis in each tube against the corresponding concentration of salt solution In normal subjects an almost symmetrical curve of sigmoid shape is obtained (Fig 20) In disease however deviations

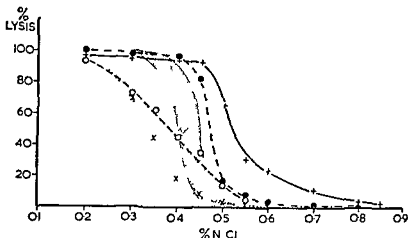


FIG 20 Erythrocyte osmotic fragility curves of patients suffering from (a) sickle cell disease (x x) (b) Mediterranean anæmia (o ——— o) (c) hereditary spherocytosis (● — — ●) and (d) idiopathic acquired hæmolytic anæmia (warm auto-antibody type) (+ ——— +) The shaded area represents the normal range

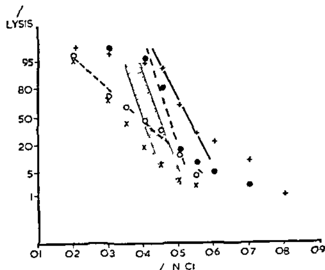


FIG 21 As Fig 20 except that the observations on osmotic fragility have been plotted on arithmetical probability paper

from the normal type of curve are found the curves for instance may have long tails due to the presence of a small proportion of very fragile cells or intermediate forms may be found The tailed type of curve is commonly found in cases of hereditary spherocytosis before splenectomy (Dacie 1943)

Two other simple alternative methods of recording the results quantitatively are available the data may be plotted on probability paper (Hunter 1940 Parpart *et al* 1947 Crawford Cutbush and Mollison 1953) or increment hæmolysis curves can be drawn (see below) Both methods emphasize any heterogeneity in the osmotic fragility of the cell population should this be present If the observed amounts of hæmolysis are plotted on the probability scale against concentrations of saline an almost straight line can be drawn through the points in the case of normal blood there being skewness only where hæmolysis is becoming almost complete (Fig 21) This method enables the MCF to be read off with ease In disease tailed curves result in varying degrees of skewness at the other end of the probability plot as well (Crawford *et al* 1953) (Fig 21)

Increment hæmolysis curves were drawn by Momigliano-Levi and Bairati (1935) Suess *et al* (1948) and by Bolton (1949) (Fig 22) With this method the differences in hæmolysis between adjacent tubes are plotted against the corresponding saline concentrations definitely bimodal curves may be obtained for instance during recovery from a hæmolytic episode Emerson and co workers (1956) have used a modified increment hæmolysis plot in extensive studies on erythrocyte osmotic fragility in hereditary spherocytosis They plotted the increment of hæmolysis on an arithmetical scale as ordinate against the corrected tonicity as abscissa using a negative logarithmic scale By this means an almost symmetrical distribution is obtained using normal blood

The relative resistance of immature cells (reticulocytes) and mature erythrocytes has been a subject of some controversy Stephens (1941) thought that the reticulocytes of the dog were less fragile than mature cells Cruz Hahn Bale and Balfour (1941) however also working with dogs reached the opposite conclusion Using erythrocytes tagged with radioactive iron they concluded that cells newly delivered to the circulation were markedly less resistant than other older cells and that this difference disappeared in 3–4 days They also obtained an indication that very old cells might be more resistant than usual Simon and Topper (1957) have reinvestigated the problem in man also using radioactive iron as a label They concluded that two populations of young cells can be separated on the basis of their osmotic fragility the majority are more resistant but there is in addition a small fraction of fragile cells After 4 months they found that most of the tagged cells

were in the most fragile fraction Prankerd (1958) also using radioactive iron reported that young cells were slightly less fragile than old cells and that the difference was accentuated by previous incubation of the cells in their own plasma for 24 hours at 37 C

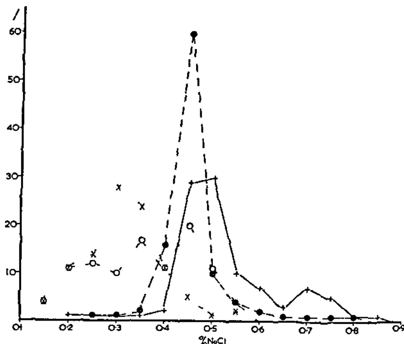


FIG 22 Increment hæmolysis osmotic fragility curves from patients suffering from (a) sickle cell disease (x x) (b) Mediterranean anæmia (o———o) (c) hereditary spherocytosis (●— — —●) and (d) idiopathic acquired hæmolytic anæmia (warm antibody type) (+———+) Drawn from the same data as are illustrated in Fig 20

Osmotic Fragility after Incubation Recently certain workers have estimated the osmotic fragility of the erythrocytes *after the blood has been incubated for 24 hours at 37° C* (*Young* 1947 Emerson Shen Ham and Castle 1947 Varadi 1951 Young Izzo and Platzer 1951 Dacie *et al* 1953 Selwyn and Dacie 1954) Under these circumstances erythrocytes from patients with hereditary spherocytosis generally undergo a greater increase in fragility than do normal cells By this procedure it may be possible to differentiate more clearly between patients with hereditary spherocytosis of the mildest degree and normal subjects

(see p 100) Dacie and his co workers (1953) found in certain congenital non spherocytic hæmolytic anæmias that the fragility

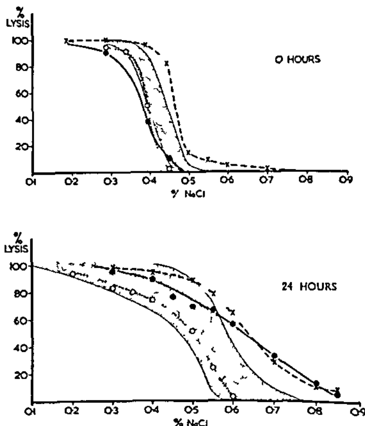


Fig 23 Erythrocyte osmotic fragility curves made from fresh blood (0 hours) and blood incubated for 24 hours at 37 C. (24 hours) The patients suffered from (*a*) hereditary spherocytosis (x— — — —x) (*b*) hereditary non spherocytic hæmolytic anæmia (Type I) (o o) and (*c*) hereditary non spherocytic hæmolytic anæmia (Type II) (●———●) respectively The shaded areas represent the normal range

might increase by an abnormal amount as the result of incubation despite the fact that the fragility test carried out before incubation gave a normal result (Fig 23) The changes following incubation

in other types of hæmolytic anæmia have been less thoroughly studied but some observations are referred to later in the relevant chapters The range of results which may be expected with normal blood when tested by the author s method (Dacie 1956) is given below (Table 3)

Table 3

Normal Range of Erythrocyte Osmotic Fragility After Incubation of the Blood for 24 hours at 37° C*

% NaCl	% Lysis
0 20	95–100
0 30	85–100
0 35	75–100
0 40	65–100
0 45	55– 95
0 50	40– 85
0 55	15– 70
0 60	0– 40
0 65	0– 10
0 70	0– 5
0 75	0
0 85	0

Median corpuscular fragility (MCF) = 0 465–0 590% NaCl

* Based on observations made on 25 healthy adult men and 21 healthy adult women
The tests were carried out at 20° C and at pH 7 4

The nature of the changes which cause diminished resistance to osmotic lysis as the result of storage of whole blood or erythrocyte suspensions *in vitro* has been closely studied Jaffe and co workers (1957) showed that the addition of various nucleosides to incubated erythrocytes increases the cells resistance to osmotic hæmolysis They concluded that their observations support the hypothesis that the maintenance of the structural integrity of the cell is dependent on the continued production and utilization of energy by the cell

Autohæmolysis

When normal defibrinated blood is incubated under sterile conditions at 37° C little or no lysis takes place in the first 48 hours thereafter autohæmolysis develops quite rapidly The exact sequences of changes which precede lysis are not yet fully known It is known however that if the glucose concentration is maintained the rate of hæmolysis is normally markedly slowed

(Selwyn and Dacie 1954) Dacie (1941) in early studies showed that in cases of hereditary spherocytosis the rate of autohæmolysis was significantly increased and might take place at 5–10 times the normal rate He found too that there was a general correlation between the osmotic fragility (of unincubated blood) and the subsequent rate of lysis on incubation the most fragile samples undergoing the most rapid lysis Blood from patients with acquired hæmolytic anæmia and spherocytosis may similarly undergo relatively rapid autohæmolysis (Dacie 1950a)

These results have been subsequently confirmed and extended in more elaborate studies by Selwyn and Dacie (1954) by Young and his co-workers (1956) and by Verloop and Bakker v Aardenne (1958) (see pp 100 and 132) The addition of glucose was found to inhibit hæmolysis markedly in hereditary spherocytosis although it did not restore the rate completely to normal in other hæmolytic anæmias the effect of additional glucose was more variable (see pp 157 and 182)

Young Izzo Altman and Swisher (1956) showed that mannose and the nucleosides adenosine guanasine and inosine were also effective in reducing the rate of hæmolysis in hereditary spherocytosis They also dealt in detail with technical factors and gave normal ranges

Some of the author s own observations are summarized in Fig 24 which illustrates the effect of glucose on the autohæmolysis of normal blood and on the blood of various types of hereditary hæmolytic anæmia The range of autohæmolysis in health after 24 and 48 hours incubation and the effect of glucose thereon based on observations made on the blood of 31 adult men and 21 adult women are tabulated below (Table 4)

Table 4

*Normal Range of Autohæmolysis**

% Lysis			
24 hours		48 hours	
Without added glucose	With added glucose	Without added glucose	With added glucose
0·05–0·5	0–0·4	0·4–4·5	0·03–0·4

* Based on observations made on 31 healthy adult men and 21 healthy adult women Defibrinated blood was incubated in 2 ml volumes under sterile conditions at 37 C Each test was carried out in duplicate

in other types of hæmolytic anæmia have been less thoroughly studied but some observations are referred to later in the relevant chapters. The range of results which may be expected with normal blood when tested by the author's method (Dacie 1956) is given below (Table 3).

Table 3

Normal Range of Erythrocyte Osmotic Fragility After Incubation of the Blood for 24 hours at 37° C*

% NaCl	% Lysis
0.20	95–100
0.30	85–100
0.35	75–100
0.40	65–100
0.45	55–95
0.50	40–85
0.55	15–70
0.60	0–40
0.65	0–10
0.70	0–5
0.75	0
0.85	0

Median corpuscular fragility (MCF) = 0.465–0.590% NaCl

* *Based on observations made on 25 healthy adult men and 21 healthy adult women.*
The tests were carried out at 20° C and at pH 7.4.

The nature of the changes which cause diminished resistance to osmotic lysis as the result of storage of whole blood or erythrocyte suspensions *in vitro* has been closely studied. Jaffe and co-workers (1957) showed that the addition of various nucleosides to incubated erythrocytes increases the cells' resistance to osmotic hæmolysis. They concluded that their observations support the hypothesis that the maintenance of the structural integrity of the cell is dependent on the continued production and utilization of energy by the cell.

Autohæmolysis

When normal defibrinated blood is incubated under sterile conditions at 37° C little or no lysis takes place in the first 48 hours; thereafter autohæmolysis develops quite rapidly. The exact sequences of changes which precede lysis are not yet fully known. It is known, however, that if the glucose concentration is maintained the rate of hæmolysis is normally markedly slowed

Spherocytosis is not the only cause of an accelerated spontaneous lysis of blood kept under sterile conditions *in vitro* In poisoning with hæmotoxic chemicals such as acetylphenyl hydrazine the same phenomenon may be observed (see Chapter 15) and this is also true of paroxysmal nocturnal hæmoglobinuria In the latter disease spontaneous hæmolysis *in vitro* is most characteristic and occurs rapidly major degrees of lysis often being visible within an hour or so It is best observed in clotted blood—hæmoglobin will be seen to diffuse from the clot into the surrounding serum—rather than in defibrinated blood or blood to which anticoagulants have been added In defibrinated blood lysis is largely inhibited by loss of carbon dioxide and consequent rise in pH and anticoagulants if in sufficient concentration may inhibit the hæmolytic reaction entirely

Crosby and Benjamin (1955, 1957) have described a further abnormal hæmolytic system leading to increased autohæmolysis This system is found in the blood of some patients with leukæmia lymphomata or other disseminated neoplastic diseases it depends apparently upon an abnormality in the plasma which brings about hæmolysis in the presence of calcium Experimentally hæmolysis was enhanced by aerating the blood raising its pH removing glucose increasing the concentration of calcium heating the serum at 60 C for 30 minutes resuspending the incubating cells after 10 hours incubation or increasing the depth of the incubating erythrocyte column (The effect of oxalate in inhibiting the abnormal hæmolysis distinguishes this system from that causing increased autohæmolysis in hereditary spherocytosis Crosby and Benjamin (1957) concluded that an erythrocyte-calcium reaction was involved and suggested that the neoplastic hæmolytic system might be a perversion or intensification of a normal response to incubation)

Van Loghem Mendes de Leon and van der Hart (1955) have further modified the autohæmolysis test so as to compare the hæmolysis of normal erythrocytes in normal serum with that in the patient s serum They state that in this way a hæmolytic factor of non immunological origin may not infrequently be demonstrated in a variety of hæmolytic anæmias and occasionally in other diseases The significance of these observations like those of Crosby and Benjamin (1957) is obscure they certainly deserve further study

An accelerated rate of spontaneous hæmolysis thus may be due to several causes amongst them spherocytosis either congenital or acquired the effects of certain chemicals and the abnormality of paroxysmal nocturnal hæmoglobinuria The phenomenon is clearly a non specific one If hæmolysis is accelerated the observer is entitled to consider it as a valuable pointer to a hæmolytic process but nothing more The heat resistance test of Hegglin and Maier (1944) is discussed in Chapter 16 in connection with the diagnosis of paroxysmal nocturnal hæmoglobinuria

The rate of autohæmolysis is most conveniently studied using defibrinated blood However, the essential differences between the behaviour of normal and pathological erythrocytes are not altered in the presence of anticoagulants (Dacie 1941 Caroli *et al* 1949)

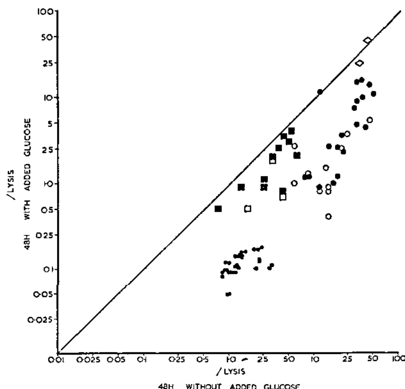


FIG 24 Effect of added glucose on autohæmolysis in hereditary spherocytosis hereditary non spherocytic hæmolytic anæmia and normal subjects

Each plot represents the amount of lysis developed after 48 hours incubation at 37 C with and without added glucose

- ● = hereditary spherocytosis before splenectomy (19 patients)
- ○ = hereditary spherocytosis after splenectomy (11 patients)
- ■ = hereditary non spherocytic hæmolytic anæmia Type I before splenectomy (11 patients)
- □ = hereditary non spherocytic hæmolytic anæmia Type I after splenectomy (3 patients)
- ◇ = hereditary non spherocytic hæmolytic anæmia Type II after splenectomy (2 patients)
- • = normal subjects (71)

useful in hereditary spherocytosis—where the mechanical fragility may be increased although the osmotic fragility test (before incubation) gives normal results in certain cases of acquired or secondary hæmolytic anæmia, and also in chronic hyperbiliru binæmia with normal liver function

SEROLOGICAL TESTS

In the acquired hæmolytic anæmias the demonstration of abnormal antibodies on the surface of the patient s erythrocytes or in his serum has proved to be of great diagnostic importance Only a brief general account of the methods of demonstrating the antibodies will be undertaken at this stage Further details are given in Chapter 9

The Direct Antiglobulin Reaction (Coombs s Test)

When auto antibodies are playing a part in bringing about erythrocyte destruction *in vivo* it is probable that the patients washed erythrocytes will nearly always if not always be found to be agglutinated by an anti human globulin serum *i.e* the direct antiglobulin (Coombs) test will be positive This test was introduced by Coombs Mourant and Race (1945) as a method for detecting incomplete Rh antibodies The same principle had been employed many years previously by Moreschi (1908) who demonstrated that erythrocytes sensitized with heterologous sera could be agglutinated by antibodies formed against the heterologous protein This work however had been forgotten The test as re introduced by Coombs Mourant and Race detects incomplete antibodies which lack the property of causing direct agglutination *in vitro* If cells which have adsorbed this type of antibody are washed in several changes of saline so as to free them from surrounding plasma or serum then they will be found to be agglutinated if subsequently suspended in an anti human globulin serum (Coombs s serum) This can be conveniently made by immunizing a rabbit against human serum (see Dacie 1956) The antiglobulin test has proved to be a very sensitive one it is capable of detecting incomplete antibodies of many different specificities including those of acquired hæmolytic anæmia

The sensitivity of the method however creates pitfalls in interpretation The first thing that has to be said is that the antiglobulin test detects protein adsorbed to cells and that a positive reaction does not necessarily mean that the cells are coated with *antibody* protein There is for instance evidence

Lysolecithin Fragility

The measurement of the resistance of human erythrocytes to solutions of lysolecithin was used by Singer (1937 1940) and by Gripwall (1938) in the investigation of cases of hæmolytic anæmia Singer and Gripwall found that whereas the resistance to lysolecithin was definitely diminished in cases of hereditary spherocytosis it was normal in symptomatic types of hæmolytic anæmia even though osmotic resistance was diminished The test has not been widely applied The diminished resistance of hereditary spherocytes was confirmed by Maier (1947) who however observed reduced lysolecithin resistance in a patient with splenic vein thrombosis in whom osmotic fragility was almost normal Foy and Kondi (1943) found that the resistance to lysolecithin of the erythrocytes of a patient suffering from blackwater fever was diminished although the osmotic fragility was normal The significance of these observations is not known If the test really is capable of differentiating clearly between hereditary and acquired spherocytes it deserves to be more widely used

Mechanical Fragility

Normal erythrocytes are susceptible to mechanical trauma and may be readily lysed *in vitro* by shaking with glass beads Increased susceptibility to lysis has been observed in certain pathological states and if the test is performed quantitatively it can be used in the investigation of hæmatological disorders (Shen Castle and Fleming 1944 Young Izzo and Platzer 1951 Dacie *et al* 1953 Schaub and Maier 1956)

Spherocytes sickled cells and agglutinated corpuscles have been shown to have an increased susceptibility to mechanical trauma but poikilocytes do not seem to be especially fragile unless spherocytic The mechanical fragility of incubated erythrocytes is increased more or less parallel to their osmotic fragility (Matthes 1950 Young Izzo and Platzer 1951) Goldbloom Fischer Reinhold and Hsia (1953) have reported that the mechanical fragility of the erythrocytes of newborn infants is almost double that of older children or adults Wagley and Lowe (1956) found that the addition of choline esterase inhibitors did not affect the mechanical fragility of human erythrocytes

The mechanical fragility test although it provides interesting information seems hardly likely to be used as a routine laboratory method The actual technique needs careful standardization and the fact that several different types of erythrocyte abnormality lead to an increased susceptibility to mechanical trauma reduces its diagnostic value Schaub and Maier (1956) with experience of over 500 tests on patients with various diseases including blood and liver disorders concluded however that the test is particularly

amounts of human γ globulin a feature which distinguishes them from the reactions of most of the warm antibodies found in auto immune acquired hæmolytic anæmia (see Chapter 9)

In connection with positive reactions given by normal cells it should be pointed out that slowly developing weak agglutination occurring as a rule in well-diluted antiglobulin serum is not uncommon With suspensions on an opalescent tile this is not as a rule evident to the naked eye under at least 7 minutes and for practical purposes is usually ignored However the agglutination is probably real and appears to represent an interaction between globulin (of uncertain nature) normally adsorbed to the erythrocyte surface and the antiglobulin serum

Boursnell Coombs and Rizk (1953) using antibodies tagged with [131]I showed that the amount of iodine non specifically adsorbed might exceed that adsorbed as the result of the specific combination of Rh positive cells and anti D antibody although the latter reaction alone gave a strongly positive antiglobulin test. Stratton and Richardson Jones (1955) have shown that diluted antiglobulin serum will agglutinate normal erythrocytes in a capillary tube inclined at an angle of 45 and that this agglutination can be abolished by either first absorbing the diluted serum with a large volume of normal cells or by adding γ globulin to it They concluded that a globulin like antigen is normally present on the surface of normal erythrocytes It is presumably this antigen which is responsible for most of the false positive reactions given by normal cells using the orthodox tile method of carrying out the antiglobulin test

Stratton and Renton (1955) have emphasized yet another possible cause of false positive agglutination This is the agglutination of red cells by silica sol derived from glass This type of agglutination is most commonly produced by using sodium citrate solutions autoclaved in glass It will of course produce agglutination of the cells in the saline control as well perhaps of the cells suspended in diluted antiglobulin serum

Falsely negative reactions may be due to three main causes the antiglobulin serum may be relatively impotent and only capable of detecting strongly sensitized corpuscles the corpuscles to be tested may have been insufficiently washed free from surrounding plasma or serum and the antiglobulin serum may have been used at an inappropriate dilution It is wrong to suppose that the dilution of an antiglobulin serum which will give an optimum reaction with say erythrocytes sensitized with anti D will react equally well with corpuscles exposed to other antibodies Cold antibodies will under certain circumstances affect erythrocytes so that they are agglutinated by antiglobulin sera (see Chapter 9) The reaction then takes place best in high concentrations of the serum and may fail to occur in serum diluted 1 in 64 or 1 in 128 concentrations which nevertheless may cause maximum agglutination of corpuscles sensitized with anti D

that cells damaged by certain drugs or chemicals (Muirhead Groves and Bryan 1954 Jandl and Simmons 1957) or cells which have adsorbed complement (Dacie Crookston and Christen son 1957) are agglutinated by antiglobulin serum Using what appear to be well absorbed sera positive tests too may be obtained occasionally with blood samples from patients suffering from a variety of diseases or even from healthy subjects (see below) In some of these instances at least it seems probable that the cells are not coated by antibody protein It cannot be assumed therefore that a positive direct antiglobulin test necessarily indicates that a patient is suffering from auto immune hæmolytic anæmia

One type of positive reaction is due to sensitization occurring *in vitro* If for instance clotted or defibrinated normal blood is allowed to stand in a refrigerator at 4° C and the antiglobulin test is subsequently carried out on cells obtained from the chilled blood the reaction may be positive due to adsorption of incomplete cold antibodies normally present in human sera (Dacie 1950b) Cells obtained from chilled oxalated or heparinized blood are less likely to give this type of positive reaction as the presence of anticoagulants inhibits sensitization

Sensitization by cold antibodies is however not the only cause of unexpected positive antiglobulin reactions In some instances the reaction will be found to be positive even if the possibility of chilling *in vitro* has been excluded by collecting the patient s blood directly into saline warmed to 37 C The cause of this type of non specific reaction is obscure It is rare to get clearly positive reactions with blood from a strictly healthy person they are however not uncommon in a variety of chronic diseases such as rheumatoid arthritis disseminated lupus erythematosus leukæmia myelosclerosis sarcoid and aplastic anæmia In some of these patients it may be possible to prove by erythrocyte survival studies that the life span of their erythrocytes is reduced but this does not seem to be true of all Although abnormal amounts of globulins are often found in the serum of these patients it does not seem to be possible to correlate the incidence of positive reactions with the presence of abnormal amounts of any particular type of globulin In particular the reaction is usually negative in patients with hepatic cirrhosis and multiple myeloma despite great increase in gamma globulins

The non specific reactions referred to in the preceding paragraph are usually weak ones maximal in high concentrations of a potent antiglobulin serum The reactions are usually relatively insensitive to the addition to the antiglobulin serum of small

and possibly *vice versa* The results obtained by the albumin method usually parallel those obtained by the indirect antiglobulin reaction

As will be referred to in later chapters patients suffering from acquired hæmolytic anæmia not uncommonly develop immune iso antibodies following transfusions These have to be taken into account in investigating the patient s serum for the presence of auto antibodies Moreover some of the auto antibodies developed by patients have definite specificities In the detection and accurate characterization of a patient s antibodies it is desirable to have available therefore a panel of normal blood samples of known genotype with which to test his serum or eluates made from his erythrocytes An important preliminary step is to determine the patient s own genotype before he receives any transfusions

Other Serological Tests

The most important test relevant to the diagnosis of hæmolytic anæmia that has not yet been mentioned is the acidified serum test (Ham s test) used in the diagnosis of paroxysmal nocturnal hæmoglobinuria (PNH) The aim of this simple test is to see whether the patient s corpuscles undergo rapid hæmolysis at 37° C in normal serum acidified to a pH between 6 5 and 7 0 When carried out with certain essential controls a positive test appears to be specific for the PNH erythrocyte abnormality

THE ESTIMATION OF THE LIFE SPAN OF ERYTHROCYTES AS A METHOD OF INVESTIGATING HÆMOLYTIC ANÆMIAS

The quantitative measurement of erythrocyte life span has proved to be of the greatest value in the diagnosis of hæmolytic anæmia and in the understanding of the mechanism of abnormal hæmolysis Although the importance of such measurements has been appreciated for a long time it is only comparatively recently that the accuracy of the available techniques has been im proved With the advent of isotopes new methods have been introduced

Two methods have proved to be particularly practicable and useful Ashby s method of differential agglutination and the tagging of erythrocytes with radioactive chromium (^{51}Cr) Ashby s method is now no longer widely used as compared with ^{51}Cr but in its day (1940–53 approximately) it yielded most valuable

For this reason it is always wise to carry out the antiglobulin test using a range of dilutions of the rabbit anti human globulin serum

Unexpected negative reactions are sometimes found in patients who otherwise seem to be suffering from hæmolytic anæmia of the auto antibody type It is conceivable that in some of these patients auto antibodies are being formed but they are of such a nature that the tests at present available fail to detect them Evans and Weiser s (1957) remarkable observation that in one patient a rabbit antiglobulin serum prepared against the *patient s serum* caused the agglutination of the patient s erythrocytes whilst other antiglobulin sera failed to do so may be quoted as an example of the inadequacy of routine methods of investigation in a particular instance Whether this observation provides the explanation for other failures remains to be seen

Detection of Antibodies in Patients Sera

Various methods are available and an outline only will be attempted here (Further details are given in Chapter 9)

Spontaneous auto agglutination occurring at room temperature or at 37° C is a pointer to the presence of abnormal antibodies

Complete (in saline agglutinating) auto antibodies may be titrated using normal corpuscles of the same blood group as the patient or normal group O corpuscles It is useful to carry out such titrations at various temperatures between 4° C and 37° C as most complete antibodies found in the sera of patients with hæmolytic anæmia excluding immune iso antibodies are cold ones

Hæmolytic antibodies can be detected in certain sera using either normal corpuscles in patients sera acidified to between pH 6 5 and 7 0 (except with antibodies of the Donath Landsteiner type which react best in unacidified serum) or by the use of enzyme treated normal erythrocytes or paroxysmal nocturnal hæmo globinuria (PNH) erythrocytes As the majority of hæmolytic antibodies are cold ones sensitization should be carried out at 20° C as well as 37 C

Incomplete antibodies can be detected in at least three ways by means of the indirect antiglobulin (Coombs) test the sensitizations being carried out usually at 37 C by the use of enzyme treated corpuscles and by titration in an albumin medium rather than in saline The first two techniques are particularly useful Both should be carried out as the results obtained are complementary some antibodies being better detected by the enzyme technique

The general accuracy of the results of these early transfusion experiments has been confirmed subsequently in many centres throughout the world It has been found too that in other hereditary hæmolytic anæmias such as sickle cell disease hereditary elliptocytosis and hereditary non spherocytic hæmolytic anæmia also due apparently to intrinsic corpuscular defects and in paroxysmal nocturnal hæmoglobinuria normal corpuscles survive normally in the patients whilst the patients corpuscles

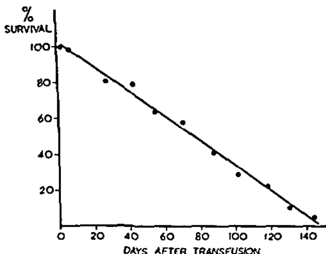


FIG 25 Survival of normal erythrocytes transfused to a patient suffering from hereditary non spherocytic hæmolytic anæmia (Type II) (Case 1 of *Dacie et al* 1953) The normal corpuscles were eliminated in a linear fashion and survived well over 100 days.

are more or less rapidly eliminated when transfused to normal recipients

In the disorders referred to in the preceding two paragraphs the rate of elimination of the normal corpuscles from the circulation of hæmatologically normal recipients has been slow and uniform about 1% or a little less of the corpuscles disappearing every day When the results are plotted on graph paper the course of elimination is almost straight *i e* linear (Fig 25) This is consistent with the idea that the life span of normal erythrocytes is comparatively constant and that elimination from the circulation in health is a function of the age of the cells alone (but see below)

information Much information on the development of methods for the study of erythrocyte life span is given by Mollison (1956a 1959) and Strumia (1956)

Ashby s Method

The differential agglutination method introduced by Ashby (1919) as a means of studying the survival of the erythrocytes of one human subject after transfusion into another consists of the transfusion into the circulation of the recipient of erythrocytes which are compatible but which are nevertheless of a serologically different group For example group O corpuscles might be transfused to a group A or AB recipient and group ON corpuscles into a group OM or OMN recipient If blood taken from the recipient after transfusion is then suspended in a potent agglutinating serum active against the recipient s cells—in the examples quoted anti A or anti M respectively—then the un agglutinated cells will be very largely those of the donor If known dilutions of blood are made in the agglutinating serum and if the procedure is carried out carefully and in a standard way then the actual numbers of unagglutinated cells per cu mm of blood may be estimated quite accurately

Ashby s method was not widely applied until the Second World War Then it was employed as a means of assessing the relative value of anticoagulant solutions in the preservation of blood (Mollison and Young 1940 1942) Dacie and Mollison (1943) modified the technique by introducing the idea of centrifuging the erythrocyte suspensions in the agglutinating serum and thus enhancing agglutination They applied the method to the study of six patients with hereditary spherocytosis in five of the patients the transfused normal blood survived for a normal length of time *i e* 100–120 days in the sixth patient elimination was complete in 60 days—this patient who was Rh negative was subsequently found to have been given inadvertently Rh positive blood The blood of one of these patients was transfused to a normal recipient In striking contrast to the normal survival of normal blood transfused to the patients this patient s blood survived both before and after the patient had undergone splenectomy for only a short time in the normal recipient Loutit and Mollison (1946) and Mollison (1947) reported observations on patients suffering from various types of acquired hæmolytic anæmia they showed conclusively in complete contrast to the results obtained in hereditary spherocytosis that these latter patients might eliminate transfused normal erythrocytes extremely rapidly

Berlin (1951) reported his more extensive survival studies The curves were practically linear in eight men but convex upwards in one man and convex downwards (curvilinear) in another In nine women believed to be in good health the curves were regularly step-wise there appeared to be a fall in the inagglutinable count in the premenstrual period compensated for at the time of menstruation by a diminished rate of destruction Hurley and Weisman (1954) illustrated two elimination curves obtained in two healthy male recipients one was definitely slightly curvilinear the other possibly so Eadie Brown and Curtis (1955) who transfused six male recipients with blood from two donors found that the blood of one of the donors was eliminated in a slightly curvilinear fashion in two of the recipients but not in the third The blood of the second donor however gave linear curves in each of the three recipients up to about 100 days thereafter elimination was slowed giving rise to a tail

The cause of this departure from the expected linearity is unknown Mollison (1956a) discussed three possibilities

(1) A flattening at the end of the curve (tailing) would be expected to be the natural consequence of variation around the mean of the life span of the erythrocyte population (This is a theoretical and reasonable explanation almost impossible to prove by the differential agglutination method) (2) It seems likely that in health a small population of short lived cells may be produced This would produce curvature at the start of the elimination curve This too has proved difficult to be certain about using differential agglutination but ⁵¹Cr studies lend some support for the suggestion (3) There may be even in health in some people some true random destruction of erythrocytes irrespective of their age If so the mechanism is unknown In recipients receiving normal donor blood the possibility of minor serological incompatibility is difficult to exclude altogether The data of Callender Powell and Witts (1947) do not suggest that menstrual loss can be the sole explanation of curvilinear elimination in women

The difficulties of exact interpretation and the complexities which result when attempts are made to analyse the form of elimination curve mathematically are illustrated in the papers of Brown *et al* (1944) Callender Powell and Witts (1947) Dornhorst (1951) Sheets Janney Hamilton and DeGowin (1951) Evans Amatuzio and Ebert (1952) Eadie and Brown (1953) DeGowin *et al* (1954) and Eadie Brown and Curtis (1955)

Recording Results of Transfusion Studies There are various ways in which the data obtained by means of a survival study may be expressed the *end point* of elimination or the *half life* (the time at which 50% of the transfused erythrocytes have been eliminated) may be recorded or the *mean cell life* calculated

The *end point* of elimination is difficult to determine with accuracy unless a *very large transfusion has been given However* when the plot of the elimination results takes the form of a straight line it is permissible to extend this to cross the time axis (abscissa)

In the acquired hæmolytic anæmias not only is the rate of elimination greatly accelerated but the course of elimination is also different when plotted on graph paper the disappearance of the corpuscles is at first rapid and then gradually slows (Fig 26) This curvilinear type of elimination was first referred to as

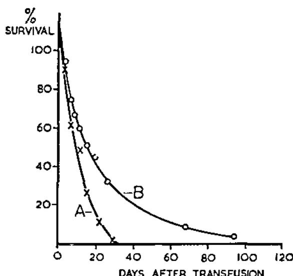


FIG 26 Survival curves plotted on ordinary graph paper of normal erythrocytes transfused to a patient (Case 13 of Dacie 1954) suffering from idiopathic acquired hæmolytic anæmia (cold antibody type) A = curve before splenectomy B = curve after splenectomy

It is possible to calculate the mean cell life from a knowledge of the area under the elimination curve if hæmolysis is complete in 30 days or less (Dornhorst 1951) Applying the calculation to Curve A

The total area = 277 square graph paper units
Initial height = 50 units

$$\frac{\text{Area}}{\text{Height}} = 5\ 5 \text{ units}$$

5 5 units on graph paper = 11 days
mean cell life = 11 days

exponential by Brown Hayward Powell and Witts (1944) It has generally been interpreted as being due to a random form of destruction in which the age of the corpuscles is unimportant

The curve of elimination is probably not always strictly linear in health Callender Powell and Witts (1947) for instance studied four healthy women and observed slightly curvilinear elimination Later

however that this method of calculation is only applicable to strictly linear elimination curves

When normal blood is transfused to a subject with an abnormal hæmolytic mechanism either of two simple methods can be used to calculate the mean cell life If the elimination is occurring in a random fashion irrespective of the age of the donor s cells the mean cell life can be ascertained by plotting the numbers of erythrocytes surviving on a logarithmic scale against time on a linear scale A straight line should fit the experimental observations and the mean cell life can be obtained by dropping a perpendicular to the time axis from the point where 3"% of the transfused corpuscles remain undestroyed (Dornhorst 1951) (Fig 27) Alternatively where the rate of elimination is fast enough for the effect of ageing to be unimportant (e g the elimination is completed in less than 30 days) the mean cell life can be estimated by dividing the area covered by the survival curve drawn on ordinary arithmetical graph paper by the initial height of the curve (Dornhorst 1951) (Fig 26) In patients in whom the transfused cells are not eliminated within 30 days a correction for ageing should be introduced This is most simply accomplished by multiplying the observed estimates of per cent cell survival by $\frac{110}{110-t}$ where t = the time after transfusion at which the estimate is made and 110 the mean cell life span of normal blood (Mollison 1950a) The corrected estimates are then plotted on squared graph paper and the area under the curve between 0 and 110 days is then measured and divided by the initial height

When congenitally defective corpuscles are transfused to normal recipients a different method of calculation has to be used Mathematically it can be shown that the mean life span of a population of erythrocytes of different life expectancies can be obtained by reading off on graph paper the intercept made by the tangent to the disappearance curve at zero time (Mills 1946 Dornhorst 1951 Crosby and Akeroyd 1952) (Fig 28)

Modifications of Ashby s Method

A sedimentation differential agglutination test has been evolved Instead of counting the numbers of unagglutinated cells after transfusion Stats (1950) measured the height of the sedimented agglutinated cells after the mixture of donor s and recipient s cells had been allowed to stand in a sedimentation tube The proportion of agglutinated cells could be calculated from the height of the column of the sedimented

and to take this as representing the end point Even so some inaccuracy is inevitable as the termination of the elimination plot is probably normally curved due to variation in the normal life span of the cell population (Dornhorst 1951) A simple alternative is to determine the *half life* of the transfused corpuscles by reading

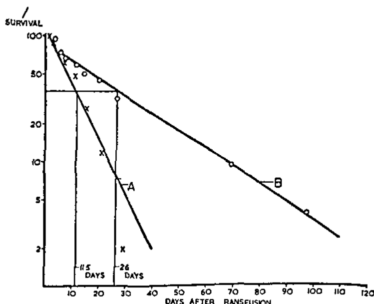


FIG 27 Same patient as in Fig 26 Survival data plotted on semilogarithmic paper A = before splenectomy B = after splenectomy

Straight lines have been fitted to the data and perpendiculars dropped corresponding with the survival of 37% of the transfused corpuscles The perpendiculars cut the time axis at the mean cell life (Dornhorst 1951)

Mean cell life (A) = 11 5 days (*cf* Fig 26)

Mean cell life (B) = 26 days

from the graph of elimination the time at which 50% of the transfused corpuscles have disappeared It is probably preferable however to calculate the *mean cell life* if attempts are made to correlate rates of cell destruction with for instance pigment excretion (Crosby and Akeroyd 1952) In health the mean cell life is given approximately by the point at which a line connecting the experimental points cuts the time axis *i e* the mean cell life is the same as the end point of elimination It must be stressed

with ^{15}N was ingested it was possible to calculate the mean life span of the erythrocytes by analysis of the isotope concentration/time curve of hæmin (Shemin and Rittenburg 1947) A figure of 127 days for the life span was obtained in one normal subject London Shemin West and Rittenburg (1949) subsequently studied the ^{15}N concentration/time curves of hæmin in patients suffering from several different blood disorders In a patient with sickle cell anæmia the half life of the erythrocytes was calculated to be 29 days and in a patient with pernicious anæmia the mean cell life was estimated to be 85 days before treatment and 129 days after treatment These results are of great interest and have the advantage of being studies of the patients own corpuscles in their normal environment However the method needs complex and expensive apparatus and materials and the data for erythrocyte survival are not more accurate than can be obtained by the Ashby or ^{51}Cr methods On the other hand the ^{15}N method has the advantage of being capable of providing at the same time valuable information on bile pigment metabolism as well as on the life span of the erythrocytes (London West Shemin and Rittenberg 1950 Gray Neuberger and Sneath 1950)

Erythrocytes previously tagged with ^{15}N in one subject have been transfused to another recipient (Watson James *et al* 1953) This type of experiment also gives simultaneous information on the disappearance of the tagged erythrocytes and the appearance of tagged stercobilin in the fæces In one experiment ^{15}N was first observed in the fæces of the recipient seventy days after the ingestion of ^{15}N-containing glycine by the donor The concentration of ^{15}N in the stercobilin reached its peak on the 122nd day

Radioactive Iron (^{59}Fe) One or other of two isotopes of iron ^{55}Fe or ^{59}Fe has been used in determining the survival of erythrocytes after transfusion (Ross and Chapin 1943 Gibson *et al* 1947) The first step in the method is to give to the donor a small amount of radioactive iron usually ^{59}Fe with a relatively short half life (47 days) This radioactive iron is incorporated in newly synthesized hæmoglobin The donor is bled and the recipient transfused when a sufficient time has elapsed for the donor s peripheral blood to have acquired the required degree of isotope activity (the maximum concentration is attained about 21 days after the administration of the isotope) The decline in the radioactivity of the recipient s blood can then be measured This technique suffers from the disadvantage that radioactive iron liberated from destroyed transfused erythrocytes is more or less quantitatively re utilized for the synthesis of fresh hæmoglobin The method is thus useless in determining the end point of elimination of the transfused corpuscles in cases where compensatory erythropoiesis is active On the other hand it can give valuable results where lysis takes place very rapidly *e g* in determining the immediate survival of stored blood (Gibson *et al* 1947)

cells using a calibration graph made for each agglutinating serum This method can be applied in exchange transfusions to determine approximately the proportion of agglutinable recipient s cells remaining A minor modification of the Ashby method was introduced by Hurley and Weisman (1953) and Hurley Weisman and Pasquariello (1954) who deliberately used hæmolytic anti A or anti B sera They claimed that the counting of the unaffected donor cells was facilitated by the removal of the recipient s cells by lysis rather than by agglutination

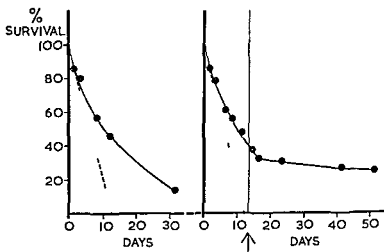


FIG 28 ^{51}Cr erythrocyte survival curves after correction for *chromium elution in two patients with hereditary spherocytosis* The patients own cells were labelled and the mean cell life span has been estimated by drawing a tangent to the initial slope of the curve The tangent cuts the base line at the mean cell life (Dornhorst 1951) in both cases this is approximately 12 days The right hand chart shows the effect of splenectomy (arrow and vertical line) The rate of hæmolysis was unaltered for about 3 days and then became approximately normal

An almost similar method was employed by Eadie Brown and Curtis (1955) They used human anti A sera reinforced by an equal volume of guinea pig serum as a source of complement Their work includes a comparative study of erythrocyte survival in normal recipients using *both the differential hæmolysis and radioactive chromium methods*

Methods Employing Isotopes

Heavy Nitrogen (^{15}N) Shemin and Rittenburg (1945) using glycine labelled with ^{15}N demonstrated that the amino acid was used in the formation of protoporphyrin from which hæmoglobin was derived Later they were able to show that if glycine labelled

(Sutherland and McCall 1955 Hughes Jones and Mollison 1956) (Fig 30) The in vivo technique is however unsatisfactory in practice as only about 10% of the injected chromium is taken up by the erythrocytes

Data on erythrocyte survival in rats and rabbits using ^{51}Cr have been published by Donohue and co-workers (1955) and by Stohlman and Schneiderman (1956) in dogs

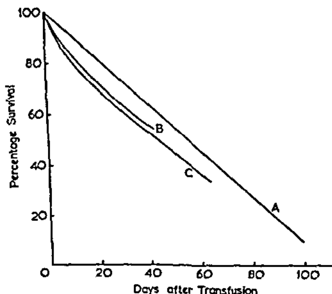


FIG 30 Comparison of Cr erythrocyte survival curves corrected for chromium elution (half period 64 days) with the usually accepted normal curve of the survival of transfused erythrocytes (A) Curve B is derived from data obtained by labelling erythrocytes in vivo by the intravenous injection of radioactive sodium chromate Curve C is derived from the curve shown in Fig 29

Reproduced by permission from Mollison (1956 Fig 23)

Practical Interpretation of ^{51}Cr Curves

Mollison (1956a) suggested that the simplest course to follow until the cause of the initial curvature of the ^{51}Cr curve in health has been determined is to assume that the linear slope represents the true survival The presence of 48–54% of the ^{51}Cr at 25 days may be taken to indicate normal survival Only when the loss of radioactivity is greater than this can increased hæmolysis be postulated The next step is to correct the observed radioactivity

observed radioactivity is corrected for chromium elution on the above basis the curve in health is still not quite linear on arithmetical paper The early part is slightly curved (Fig 30 reproduced from Mollison (1956a))

The linear part of the curve cuts the ordinate somewhere below 100% according to Mollison and Veall (1955) at 94% This suggests that loss of radioactivity from the circulation (due to

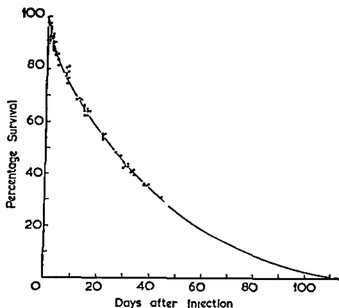


Fig 29 Rate of disappearance of Cr from the circulation in 11 normal subjects after the injection of the subjects own erythrocytes labelled with Cr
Reproduced by permission from Mollison (1956 Fig 2)

the combined effects of elution and hæmolysis) normally occurs in two phases an initial relatively rapid phase lasting 2–3 days and a subsequent slower phase The cause of the initial loss of radioactive chromium has been the subject of much discussion but a wholly satisfactory explanation is still awaited (see Mollison 1956a Hughes Jones and Mollison 1956)

Erythrocytes have also been labelled with ^{51}Cr *in vivo* by injecting the radioactive sodium chromate *intravenously* *It is interesting to* note that the form of the elimination curve of radioactivity is the same irrespective of whether the labelling is carried out *in vivo* or *in vitro*

^{51}Cr survival curves in acquired hæmolytic anæmia hereditary elliptocytosis and paroxysmal nocturnal hæmoglobinuria are illustrated in Fig 31

Effect of Blood Volume Changes on the Estimation of Erythrocyte Survival

The extra difficulty in the interpretation of ^{51}Cr survival curves when changes in erythrocyte volume plasma volume or both occur during the course of the study has been stressed by Strumia and co-workers (1955) Turnbull Hope and Verel (1957) and Birkeland (1958) (Normally when measuring blood radioactivity it is assumed that as the tagged cells disappear their place is taken by newly formed cells or plasma so that the total blood volume is not altered If the total blood volume in fact diminishes parallel with the disappearance of the tagged cells measurement of the radioactivity of the remaining blood will overestimate their survival If on the other hand there is over production of plasma so that the total blood volume increases the measured level of radioactivity will be erroneously low and erythrocyte survival will be underestimated)

Some workers have recommended measuring the radioactivity of packed erythrocytes rather than that of whole blood but the accuracy of this technique depends too on the replacement of the tagged cells by a similar volume of new cells As Turnbull Hope and Verel (1957) point out in normal subjects or in patients who are similarly in a steady state no corrections are necessary Correction to the original packed cell volume level only increases accuracy if any subsequent deviations are due to alterations in plasma volume alone for if the erythrocyte volume falls and the plasma volume increases correspondingly then correction to the original packed cell volume will introduce a large error When genuine erythrocyte volume changes occur and lead to changes in the total blood volume as in patients recovering from blood loss or being treated for iron deficiency only a re estimation of the total blood volume using ^{32}P or Evans blue dye can provide data on which accurate compensation can be based

In vivo Counting after the Injection of ^{51}Cr Labelled Erythrocytes

In the last few years the estimation of the survival of ^{51}Cr tagged erythrocytes has been combined with in vivo counting using a scintillation counter over organs such as the spleen and liver (Korst Clatanoff and Schilling 1955 Jandl *et al* 1955 Motulsky Casserd and Giblett 1956 Jandl *et al* 1956 Hughes Jones and Szur 1957 Harris McAlister and Prankerd 1957 Jandl Richardson Jones and Castle 1957 Schloesser *et al* 1957 Mollison and Hughes Jones 1958 McCurdy and Rath 1958) It is already clear that this technique is capable of indicating which are the main sites of hæmolysis in patients with hæmolytic anæmia This information has a theoretical importance in relation

for elution of chromium by plotting on semilogarithmic paper a line linking 50% radioactivity at 70 days to 100% radioactivity at 0 days The experimental estimates are then each multiplied by the appropriate fraction *e g* at 70 days by $\frac{100}{50}$ and at 20 days by $\frac{100}{82}$ etc The corrected estimates can then be plotted on semilogarithmic paper If derived from a patient with acquired hæmolytic anæmia the points will probably fit a straight line

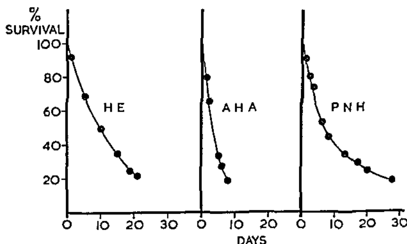


FIG 31 Cr erythrocyte survival curves after correction for chromium elution in patients with hereditary elliptocytosis (HE Case 1 p 161) idiopathic acquired hæmolytic anæmia (warm antibody type) (AHA) and paroxysmal nocturnal hæmoglobinuria (PNH)
The patients own cells were labelled.

The mean cell life then corresponds to the time at which there is 37% survival as already discussed (p 57) If the data are derived from a patient with a hereditary hæmolytic anæmia the corrected estimates should be plotted on arithmetical paper and the tangent to the initial slope drawn (see p 57) This is also probably the best thing to do in acquired hæmolytic anæmia if a straight line will not fit the data when they are plotted on semilogarithmic paper

The normal values for ^{51}Cr remaining in the circulation from 1 to 44 days after injection are given by Mollison (1956 p 139)

^{51}Cr survival curves in acquired hæmolytic anæmia hereditary elliptocytosis and paroxysmal nocturnal hæmoglobinuria are illustrated in Fig 31

Effect of Blood Volume Changes on the Estimation of Erythrocyte Survival

The extra difficulty in the interpretation of ^{51}Cr survival curves when changes in erythrocyte volume plasma volume or both occur during the course of the study has been stressed by Strumia and co-workers (1955) Turnbull Hope and Verel (1957) and Birkeland (1958) (Normally when measuring blood radioactivity it is assumed that as the tagged cells disappear their place is taken by newly formed cells or plasma so that the total blood volume is not altered If the total blood volume in fact diminishes parallel with the disappearance of the tagged cells measurement of the radioactivity of the remaining blood will overestimate their survival If on the other hand there is over production of plasma so that the total blood volume increases the measured level of radioactivity will be erroneously low and erythrocyte survival will be underestimated)

Some workers have recommended measuring the radioactivity of packed erythrocytes rather than that of whole blood but the accuracy of this technique depends too on the replacement of the tagged cells by a similar volume of new cells As Turnbull Hope and Verel (1957) point out in normal subjects or in patients who are similarly in a steady state no corrections are necessary Correction to the original packed cell volume level only increases accuracy if any subsequent deviations are due to alterations in plasma volume alone for if the erythrocyte volume falls and the plasma volume increases correspondingly then correction to the original packed cell volume will introduce a large error When genuine erythrocyte volume changes occur and lead to changes in the total blood volume as in patients recovering from blood loss or being treated for iron deficiency only a re-estimation of the total blood volume using ^{32}P or Evans blue dye can provide data on which accurate compensation can be based

In vivo Counting after the Injection of ^{51}Cr Labelled Erythrocytes

In the last few years the estimation of the survival of ^{51}Cr tagged erythrocytes has been combined with in vivo counting using a scintillation counter over organs such as the spleen and liver (Korst Clatanoff and Schilling 1955 Jandl *et al* 1955 Motulsky Casserd and Giblett 1956 Jandl *et al* 1956 Hughes Jones and Szur 1957 Harris McAlister and Prankerd 1957 Jandl Richardson Jones and Castle 1957 Schloesser *et al* 1957 Mollison and Hughes Jones 1958 McCurdy and Rath 1958) It is already clear that this technique is capable of indicating which are the main sites of hæmolysis in patients with hæmolytic anæmia This information has a theoretical importance in relation

to the pathogenesis of the hæmolysis and a practical importance in relation to the possibility of benefit from splenectomy (see p 187) Details are given later when the different types of hæmolytic anæmia are described but in brief it can be said that the results to date have shown that in disorders where splenectomy is known to be of clinical value there is a marked uptake of radio active chromium by the spleen as compared with the uptake of chromium by the liver and conversely in disorders in which

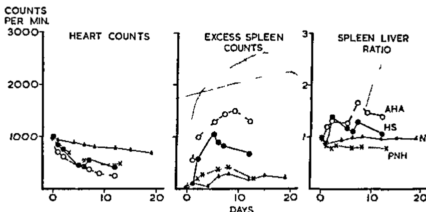


FIG 32 Results of in vivo surface counting after the labelling of patients erythrocytes with ^{51}Cr The figure shows the heart counts the excess of spleen counts over the heart counts and the ratio of the excess spleen counts to the excess of liver counts (spleen liver ratio)
AHA = a patient with idiopathic acquired hæmolytic anæmia (warm antibody type) HS = a patient with hereditary spherocytosis N = a hæmatologically normal subject PNH = a patient with paroxysmal nocturnal hæmoglobinuria

splenectomy has no favourable effect the uptake by the spleen barely exceeds that of the liver

Typical observations in cases of hereditary spherocytosis auto immune acquired hæmolytic anæmia and paroxysmal nocturnal hæmoglobinuria are shown in Fig 32

Estimation of erythrocyte survival using ^{51}Cr combined with in vivo surface counting has also been used with great success in the investigation of incompatibility due to iso antibodies (Mollison and Cutbush 1955 Jandl Richardson Jones and Castle 1957 Cutbush and Mollison 1958 Mollison and Hughes Jones 1958) The relevance of these data to the problems of hæmolysis due to auto antibodies is considered in Chapter 11

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for instance in hereditary spherocytosis the hæmoglobin itself is normal and the abnormality seems to depend on a subtle defect in erythrocyte metabolism These fundamental problems are considered in more detail later when each type of disease is dealt with separately

From the point of view of diagnosis the presence in a patient suffering from hæmolytic anæmia of an intrinsic erythrocyte defect is indicated by the unimpaired survival in the patient of transfused normal corpuscles or by the unequivocal demonstration *in vitro* of an abnormality of the patient s corpuscles such as the presence of an abnormal hæmoglobin or less specifically by an increased tendency of the erythrocytes of the patient to undergo lysis or behave abnormally *in vitro* under certain specified conditions (see Chapter 1 pp 35–47)

HEREDITARY SPHEROCYTOSIS

Synonyms La microcythémie (Vanlair and Masius 1871) hereditare chronischer Ikterus (Minkowski 1900) L ictère congenital de l adulte (Chauffard 1907) hamolytischer Ikterus (Gansslen 1922) konstitutionelle hamolytische Anamie (Spharo cytenanamie Kugelzellenanamie) (Naegeli 1931) acholuric jaundice (Campbell 1925–26) spherocytic icterus (Krumbhaar 1936) familial hæmolytic anæmia (Dacie 1943) hereditary spherocytosis (Committee for Clarification of Nomenclature 1950)

Of the synonyms referred to above *hereditary spherocytosis* seems the most appropriate The title refers to the inherited nature of the disease as well as to a fundamental laboratory sign—spherocytosis

History The first significant contribution to the literature on hereditary spherocytosis appears to be that of Vanlair and Masius who in 1871 gave a remarkably accurate description of the disease under the title la microcythémie They recognized that some of the erythrocytes of their patient were small and spherical in character and a little more deeply coloured than normal and suggested that they were corpuscles on their way to destruction (globules atrophiques) and that excess bile pigment was derived from them The illustration from their paper is reproduced as Fig 33 Vanlair and Masius s work has not received the recognition that it deserves

No other notable contribution was made for almost 20 years Then in 1890 Wilson gave an account to the Clinical Society of London of six members of a family in whom a condition in which

CHAPTER 2

THE CONGENITAL HÆMOLYTIC ANÆMIAS

1 HEREDITARY SPHEROCYTOSIS

A FAMILIAL form of jaundice was first recognized by physicians towards the end of the nineteenth century (Murchison 1885 Wilson 1890 Wilson and Stanley 1893) Wilson and Stanley s account was particularly important and undoubtedly referred to hereditary spherocytosis the splenomegaly the jaundice of the skin and conjunctivae the periodic attacks of deeper jaundice associated with biliary colic the inherited nature of the disease its chronicity and the fact that it was compatible with a long life—all these features were well described It was left to Le Gendre (1897) and Hayem (1898) to show that this type of jaundice was *acholuric* with bile in the plasma but not in the urine In 1900 followed Minkowski s well known description of eight cases of jaundice in three generations in this paper most of the salient clinical features of hereditary spherocytosis were well described

It is now realized that the disorder (hereditary spherocytosis) so clearly described by Wilson and Stanley (1893) and by Minkowski (1900) is but one of a series of distinct types of congenital hæmolytic disease In this and the following four chapters descriptions will be given of five major types *hereditary spherocytosis hereditary elliptocytosis hereditary non spherocytic hæmolytic anæmia Mediterranean anæmia* and *sickle cell disease and allied disorders* Other less clearly defined types exist and these will also be briefly considered

All types of congenital hæmolytic anæmia depend upon an inherited abnormality of the patients erythrocytes the different diseases being distinguished by differences in the nature of the erythrocyte abnormalities and in the modes of inheritance In each type the result of the abnormalities is that the life span of the patients corpuscles is shortened All grades of cellular defect are found and this leads to marked differences in the severity of the anæmia from which the patients suffer

The nature of the erythrocyte defects is in most instances poorly understood It is known however that in sickle cell disease the molecules of hæmoglobin are abnormal and that in Mediterranean anæmia there is a defect in hæmoglobin synthesis In other types as

for instance in hereditary spherocytosis the hæmoglobin itself is normal and the abnormality seems to depend on a subtle defect in erythrocyte metabolism These fundamental problems are considered in more detail later when each type of disease is dealt with separately

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an enlarged spleen accompanied by a sallow or subicteric complexion appears as an hereditary condition ' Next a further and more detailed account was given by Wilson and Stanley (1893) and anæmia was recognized as an important feature of the disease Significantly they concluded that no doubt can be entertained that the splenic disease is accountable for this One of the patients died her spleen was examined and found to be firm and dark red on section and microscopic sections showed *it to be engorged with blood cells* Death was considered to have been due to active hæmolysis of splenic origin

A detailed account of Wilson s papers is given by Campbell (1925–26) who examined the only survivor of Wilson s six patients and confirmed the diagnosis of hereditary spherocytosis

Wilson s descriptions were followed in 1900 by the better known report of Minkowski Minkowski s paper was soon followed by those of Gilbert Castaigne and Lereboullet (1900) in France and by that of Barlow and Shaw (1902) in England Barlow and Shaw recognized that their cases were probably similar to those of Wilson Their report is interesting in that they record the presence of ulcers on the lower part of the leg in both their patients In America hereditary spherocytosis was first described in detail by Tileston and Griffin (1910) and by Thayer and Morris (1911)

The disease has since been reported from many parts of the world and it now has a voluminous literature Reviews and monographs include those of Tileston (1922) Gansslen Zipperlen and Schuz (1925) Campbell (1925–26) Meulengracht (1922 1938) Bamatter (1932) Cheney and Cheney (1934) Gansslen (1936) Vaughan (1936) Gripwall (1938) Dacie (1943) Young Izzo and Platzer (1951) and Young (1955b) While the majority of cases of hereditary spherocytosis conform closely to a well recognized pattern there is some evidence which suggests that more than one type of the disorder may exist (see p 109) It would perhaps be surprising if this were not so in view of what is now known of other well known inherited blood diseases such as sickle cell disease

CLINICAL FEATURES

Inheritance According to Meulengracht (1921) Plate (1913) was the first to suggest that hereditary spherocytosis was inherited as a Mendelian dominant Meulengracht himself investigated seven families in Stockholm He found that the healthy members of the families did not transmit the disease and with one exception that the disorder was always inherited through an affected parent

Meulengracht attributed the exception to mutation Subsequent workers have confirmed the general truth of Meulengracht s observations (Campbell and Warner 1925–26 Race 1942 Young *Izzo and Platzer* 1951 *Abrams and Battle* 1952)

The most recent large scale detailed study is that of Race (1942) who examined 183 members of 20 different families in which the disease had occurred He confirmed that the inheritance followed a Mendelian dominant pattern although there was a deficiency in the expected number of affected siblings Race attributed this to two factors an unusually high miscarriage rate and infant mortality of affected compared with unaffected siblings and variation in penetrance leading to mild and easily missed forms of the disease In four out of the 20 families studied by Race both parents of an affected propositus were apparently unaffected in three of the families all of 16 other relatives studied were also unaffected Race considered that mutation was a possible but unlikely explanation and that the observation gave some support for an acquired form of the disease Two of Race s patients (with both parents unaffected) were studied by the author in 1938 in these at least there seemed good evidence that the disease was congenital—both propositi were small children and the disease in each was typical in every way If mutation is in fact improbable it seems that limitation of penetrance in a carrier parent is the more likely explanation It is well known for instance that the disease in an active form may be transmitted by a parent who shows only minimal signs of the disease In the studies of both Race (1942) and Young Izzo and Platzer (1951) there was no deficiency in the expected numbers of affected children of propositi although the number of affected siblings was below expectations

Young (1955a) in a more recent review of his cases has reported that out of 28 families with the disease there were five in which the blood of both parents of the propositus could not be distinguished from normal—the osmotic fragility of the erythrocytes was even normal after 24 hours incubation However in one family the blood of the maternal grandmother showed slight spherocytosis and increased osmotic fragility after 24 hours incubation and in another family there were two affected siblings Young considered that in these families the expression of the abnormal gene was extremely feeble and that a true carrier state for the gene of hereditary spherocytosis probably exists Ham (1955) in discussing Young s paper reported that amongst his families there were four in which the blood of both parents appeared to be normal or only doubtfully abnormal Significantly in one family in which the mother of the propositus may have had the disease in a just detectable form a maternal aunt had overt hereditary spherocytosis

An alternative explanation for some of the instances of sporadic hereditary spherocytosis is that they may represent examples of an atypical type of congenital spherocytosis which differs perhaps in inheritance as well as possibly in the nature of the erythrocyte defect (see p 109)

Hereditary spherocytosis has apparently not yet been studied in an unequivocally homozygous state By analogy with hereditary elliptocytosis and sickle cell disease it should then give rise to a severe

hæmolytic anæmia Race (1942) has even suggested that the gene in a homozygous state may be lethal In one of his families (No 26) two probable heterozygotes who were first cousins married Two miscarriages occurred and Race suggested that these may have been the homozygotes However Bernard Boiron and Estager (1952) have more recently reported studies on a family of 13 children all of whom suffered from varying degrees of anæmia jaundice and splenomegaly The father was also affected but the mother and her relatives were apparently normal Bernard and his colleagues considered that the most likely explanation for all 13 children being affected was that their father was homozygous for hereditary spherocytosis Unfortunately this could not be proved as clear evidence of jaundice was only found on the maternal side of his family [1]

Race and Incidence Hereditary spherocytosis is an abnormality probably not confined to any particular race However it is certainly best known as a disease affecting people of European origin It is rare in negroes but probably not as rare as was at one time thought All the cases so far recorded in negroes seem to have been discovered in America (Scherer and Cecil 1945 Goodman and Cates 1947 McCormack and Simon 1948 Butterworth Kracke and Riser 1950 Esmond Quinn and Peters 1955) Kline and Holman (1957) have recently reported six cases in one family They stated that up to the time of their report only 42 cases had been described in negroes and that these were confined to 13 families The disease has been reported by Salah (1936) in Egyptians and by Stransky and Dauis Lawas (1952) in the Philippines Its true incidence in races other than European is not known In Britain it cannot be considered a rare disease In America Young (1955b) reported that he knew of 50 cases (in 28 families) amongst a largely ex European population of about 1 000 000 in upper New York State The low mortality and the

[1] Motulsky Huestis and Anderson (1956) have published a fascinating study of hereditary spherocytosis in the deer mouse (*Peromyscus*) The disorder is in many ways similar to hereditary spherocytosis in man It is however inherited as an autosomal recessive Affected newborn mice are pale and jaundiced adult mice are not anæmic although the reticulocyte count is raised to 15 % (about eight times normal) Erythrocyte osmotic fragility is increased The spleen is enlarged and was calculated to contain about 24 times as much blood as normal Cell survival studies give results similar to those obtained in man and the survival of the pathological erythrocytes was found to be shorter in normal animals than in affected animals After splenectomy the reticulocyte count falls to almost normal levels and cell survival is also almost normal

The affected animals were homozygotes and the heterozygotes could not be identified The animals varied in their degree of jaundice and that genetic modifiers might be involved was suggested by the fact that if very jaundiced mice were mated an increased proportion of their progeny were deeply jaundiced

excellent results of splenectomy suggest that with a steady mutation rate the incidence of the disorder is likely to increase

Age and Sex The disease is not sex linked Being congenital its presence is most frequently diagnosed for the first time when the patient is a child or young adult However there are numerous exceptions to this and it is by no means uncommon for the disorder to be unrecognized in infancy and early childhood Debré Lamy Sée and Schrameck (1938) reporting on 20 cases found that whereas the disease was diagnosed in each case before the age of 14 in only three patients had the diagnosis been made before the age of 4 years Exceptionally the diagnosis may be made for the first time in an elderly subject attending hospital for some unrelated complaint or because one or more of his children have been found to be affected An extreme example of delayed diagnosis is illustrated by one of the family pedigrees published by Race (1942 Family 9) The propositus of this family was a man aged 77 who was found to have a palpable spleen and slight anæmia when attending hospital for bronchitis Subsequently two of his children and a grandson were also found to be affected they considered themselves to be healthy although in fact they had the disease in a more severe form than had the propositus

On the other hand hereditary spherocytosis has been diagnosed in infants at or within a few days of birth (Hawksley 1936 Conrad and Schmidt 1946 Macaulay 1951 Newns 1951 Bernard Boiron and Estager 1952 Roddy 1954 Shapiro Josephson Rosengvaig and Kauffman 1957 Stamey and Diamond 1957 Burman 1958) Usually this has been done when one of the parents or a previous sibling has been known to be affected and the disease has been particularly looked for In general the more severe the disease the more likely is it that the diagnosis will be made at an early age

Associated Abnormalities Gansslen Zipperlen and Schuz (1925) and Gansslen (1936) stressed the association between hereditary spherocytosis and other congenital abnormalities Gansslen thus referred to Die hamolytische Konstitution He particularly stressed the occurrence of Turmschadel (tower skull) brachycephaly eye abnormalities polydactyly brachydactyly and infantilism Hansen and Klein (1934) added other abnormalities which they thought characteristic such as arched palate broad base of nose squint and dental abnormalities and Otto (1955) has more recently reviewed a long list of eye abnormalities Other authors such as Meulengracht (1938) Gripwall (1938) and Debré and co workers (1938) have not seen associated

abnormalities with anything like the frequency that Gansslen and Hansen and Klein reported them Of Race s patients only three had gross associated abnormalities *congenital absence of* a hand in one patient cervical ribs in another and mental deficiency in another Young (1955b) too reported only a low incidence of associated abnormalities thickening of the vault of the skull in one patient with retarded mental development and otosclerosis in three members of another family It seems likely therefore that in most families the incidence of skeletal abnormalities is not higher than in the general population Nor is there any evidence for genetic linkage with sex the ABO and MN blood groups ability to taste phenylthiocarbamide ability to secrete the ABO antigens in saliva eye colour or ear lobe attachment (Race 1942)

There are a few reports of the occurrence of endocrine abnormalities in association with hereditary spherocytosis Freymann (1922) described two brothers and a sister with tower skull delicately formed bones and hypogenitalism and Curschmann (1923) two patients with signs of hypogenitalism and infantilism More recently Falconer (1936) referred to a girl with signs of ovarian hypofunction and obesity and Debré and his colleagues (1938) to an occasional tendency to backwardness in physical and sexual development As mentioned on p 86 Bernard Boiron and Estager (1952) described a family of 13 affected children in nine out of the 13 children there was clear evidence of physical and mental retardation in four of them this amounted to true infantilism They all improved remarkably after splenectomy

Under the term les syndromes neuro hemolytiques herédo familiaux French authors (*e g* Portier Massonat Garre and Natter 1954) have referred to the occurrence of signs of degenerative changes *in the nervous system in cases of hereditary hæmolytic disease including* hereditary spherocytosis thalassæmia and sickle cell disease The exact significance and frequency of the syndromes need further definition

Symptoms and Physical Signs

Patients with hereditary spherocytosis usually present with jaundice, signs of anæmia, and an enlarged spleen They may give in addition a history suggestive of gallstones Occasionally they complain of intractable leg ulcers As mentioned above some patients make no complaint the disease being discovered accidentally in others one or more of the main clinical features may be absent

Jaundice The jaundice is characteristically *acholuric* the direct Hijmans van den Bergh reaction is almost always negative and the urine contains urobilin but not bile The degree of bilirubinæmia varies usually the plasma level lies between 1 and

4 mg per 100 ml Occasionally despite other signs of active hæmolysis the plasma bilirubin level is within the normal range *i e* 0 8 mg per 100 ml or less (King and Wootton 1956) in such cases the liver is presumably particularly efficient in excreting the excess bilirubin formed Jaundice is usually not noticeable in children in the first few years of life (Debré *et al* 1938) As a rule it is not until an affected child has reached school age or adolescence that jaundice appears—the early onset in the large family of Bernard Boiron and Estager (1952) is exceptional Jaundice may nevertheless be present in the neonatal period and is commoner than has been supposed (Dawson 1931 Newns 1951 Roddy 1954 Stamey and Diamond 1957 Burman 1958)

Occasionally deep jaundice develops 24–36 hours after birth and this may even be associated with incipient kernicterus For instance an infant described by the staff of St Christopher s Hospital Philadelphia (Roddy 1954) developed a serum bilirubin concentration of 26 6 mg per 100 ml on the 4th day after birth Exchange transfusion was carried out and 12 hours later splenectomy recovery was complete An elder brother also became intensely jaundiced in the neonatal period but did not receive an exchange transfusion There were signs of opisthotonos from the 5th to 14th day and subsequently he showed some evidence of mental retardation Betke (1956) reported a further case the infant whose serum bilirubin rose to 25 4 mg per 100 ml was treated by exchange transfusion

Stamey and Diamond (1957) have more recently reported that twenty three out of 43 infants with hereditary spherocytosis gave a history of neonatal jaundice They also described four infants with deep jaundice who were successfully exchange transfused They point out the difficulty in distinguishing neonatal hereditary spherocytosis from hæmolytic disease of the newborn due to ABO incompatibility (see Chapter 17) and recommend that exchange transfusion (repeated if necessary) should be carried out if the bilirubin concentration in the serum exceeds 20 mg per 100 ml

Plasma bilirubin levels greater than 4 mg per 100 ml seem in most instances to be due not so much to an exceptional rate of hæmolysis as to the liver being relatively inefficient in excreting bilirubin Bile is occasionally found in the urine This is usually due to biliary obstruction resulting from pigment gallstones Less commonly it is found in severe hæmolytic crises in the course of *which liver damage sometimes develops* *Occasionally* darkening of the urine due to the presence of excessive amounts of urobilin or even bile pigment associated with pale stools suggests infective

hepatitis This however does not seem necessarily to be the explanation in some patients it is tempting to imagine that transient episodes of this sort could be the result of sludging of the bile in the liver canaliculæ However there seems little or no positive evidence in favour of this hypothesis

In the series of patients described by Young Izzo and Platzer (1951) *the pre operative bilirubin levels ranged from 0 6 to 5 7* mg per 100 ml the mean bilirubin level was 2 0 mg per 100 ml (see also Table 5) More recently Young (1955b) has reported a wider range (0 4–13 0 mg per 100 ml) with the mean still approximately 2 0 mg per 100 ml

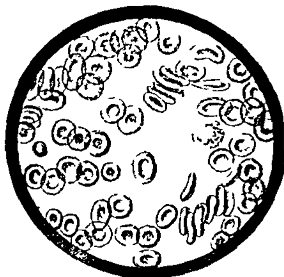
Anæmia Anæmia in hereditary spherocytosis is very variable in degree it is unusual for it to be extremely severe not uncommonly it is slight or even absent In most patients the hæmoglobin level lies between 7 5 g and 14 g per 100 ml As a rule the rates of hæmolysis and regeneration are sufficiently stable for each patient to maintain a fairly steady hæmoglobin level for long periods

Minor Crises It is characteristic of the disease that from time to time the anæmia increases the patient may then complain of abdominal pain and develop pyrexia His jaundice may deepen but it is often unaltered Occasionally as mentioned above the stools may be pale and the urine dark Vomiting is not uncommon and the spleen may increase in size In children unexplained pyrexia and tachycardia with abdominal pain and vomiting occurring at intervals and not associated with obvious jaundice may be the presenting symptoms of the disease (Debré *et al* 1938) After a few days or a week or so the exacerbation usually passes off and the patient s hæmoglobin will then rise to about its usual level Minor crises such as these often follow intercurrent infections on other occasions there appears to be no obvious cause Not infrequently the diagnosis in a mild case may be made for the first time as the result of an increase in anæmia following an infection The mechanism of these minor crises seems to be twofold some appear to be associated with an increased rate of hæmolysis others with temporary depression of erythropoiesis More serious crises occasionally develop these crises which almost always appear to be due to a sudden failure in erythropoiesis are of special interest and will be dealt with in a later section (p 110)

Splenomegaly The spleen is probably invariably enlarged and it is uncommon to find it impalpable—it was so in six out of the 29 patients of Young Izzo and Platzer (1951) In affected



I



II

Fig. 33. Reproduction of Vanlair and Masius's illustration to their paper "De la microcythémie" (*Bull. Acad. roy. Méd. Belg.*, 5, 3rd series, [illegible] 1871).

I is a drawing of the patient's blood.

II a drawing of control normal blood.

To face p. 90



FIG 31 Severe bilateral chronic ulceration of the legs in a patient with hereditary spherocytosis Female aged [illegible] (Reproduced by courtesy of Dr A Calpin)



FIG 3[illegible] Severe chronic ulceration of the left leg of a patient with hereditary spherocytosis Male aged 48 (Reproduced by courtesy of Dr A Calpin)

children a palpable spleen seems to be a particularly constant sign (Debré *et al* 1938) it may in fact be the only certain physical sign of the abnormality in a child or sibling of a known patient Usually the lower edge of the spleen is palpable somewhere between the left costal margin and the level of the umbilicus In consistency the spleen feels moderately firm occasionally it is tender on palpation It generally moves freely on respiration and at operation it is usually free and not adherent The histology of the spleen is considered on p 114

Gallstones Many patients suffering from hereditary sphero cytosis develop gallstones (Cheney and Cheney 1934 Bates and Brown 1952) They may be found even in children (Gairdner 1939) In the series of patients reviewed by Bates and Brown (1952) gallstones were present in one out of 19 children less than 10 years of age and in 39 out of 74 patients older than 10 years of age The stones are a potential source of trouble and not infrequently lead directly or indirectly to the death of the patient Sometimes the presence of the hæmolytic anæmia itself is first discovered in a surgical ward the patient having been admitted for surgical treatment of a complication of gallstones The gallstones are of the pigment variety and presumably develop as the result of the increased concentration of bilirubin in the bile If their presence leads to cholecystitis or cholangitis stones of mixed type containing bile pigment and cholesterol may form Mixed stones are radio opaque pure pigment stones are not

Leg Ulcers Intractable ulcers of the leg not associated with varicose veins are a remarkable but rather uncommon complication of hereditary spherocytosis (Figs 34 and 35) They were recorded as early as 1902 by Barlow and Shaw in a mother and her son As a rule the ulcers heal quickly after splenectomy (Vaughan 1936 Leger and Orr 1940) They are usually found in the old or middle aged patient Dedichen (1931–32) however reported crural ulcers in three young men (two of them brothers) aged 15 17 and 17 years respectively In each case the ulcers healed after splenectomy Another example in a woman aged 20 was described by Taylor (1939) Here too the ulcer healed after splenectomy The ulcers illustrated in Fig 34 had been present for 42 years They healed within 6 weeks of splenectomy but recurred 18 years later One of the ulcers has since become malignant (Edwards 1955)

The ulcers are generally bilateral and nearly always start well above the medial malleolus In severe cases they may extend almost completely round the leg and also upwards for a

considerable distance (Figs 34 and 35) They are quite indolent and are associated with pigmentation of the surrounding skin. In some patients an extensive indurated erythematous area develops rather than actual ulceration (Beinhauer and Gruhn 1957) The presence of the ulcers is not diagnostic of hereditary spherocytosis for they are not uncommon in sickle cell disease and may rarely be met with in other chronic diseases associated with splenomegaly (Gendel 1948)

Blood Picture

Erythrocytes As already referred to (p 20) the erythrocytes in hereditary spherocytosis tend to be more spheroidal and less disc like than normal corpuscles The mean diameter of the cells is less than normal and their breadth (thickness) greater than normal the normal biconcavities are less marked It must be emphasized that the extent of the cellular abnormality varies from case to case and that there is in addition a considerable variation in the degree of spherocytosis in the cell population of any particular patient It seems probable that as the erythrocytes mature they become more and more spherocytic, and that the youngest cells (the reticulocytes) are the most disc like (leptocytic) Nevertheless it has been shown that the reticulocytes in hereditary spherocytosis although thin discs are less disc like and have smaller diameters than normal reticulocytes (Paulino 1949 Schrumpf 1956b) The contrast in size between the spherocytes and the less densely staining more flattened cells some of which are reticulocytes is shown in the stained blood film illustrated in Fig 37

The dimensions of the spherocytes of hereditary spherocytosis have been repeatedly studied Figures for mean cell diameter (MCD) mean cell thickness (MCT) and mean cell thickness diameter ratio are to be found in papers by Vaughan and Goddard (1934) Hawksley and Bailey (1934) Heilmeyer (1936) Hawksley (1936) Vaughan (1937) Gripwall (1938) Morgensen (1938) and Young Izzo and Platzer (1951) In five personally studied cases the average mean cell diameter (measured on dry films) was 6 4 μ (normal mean 7 2 μ) and the mean cell thickness (calculated from the MCD and MCV) was 2 6 μ (normal mean 2 μ) the average thickness diameter ratio was 0 40 (normal mean 0 28) The microcytosis (and also the increased anisocytosis) can be well demonstrated if Price Jones curves are drawn (Fig 36) Harboe and Schrumpf (1955) have demonstrated and carried out quantitative measurements of spherocytosis by estimating the extent of

the shadows cast by erythrocytes coated at an angle *in vacuo* by vaporized gold

The mean corpuscular volume is usually within the normal range before splenectomy the mean value in 42 of the writer s cases

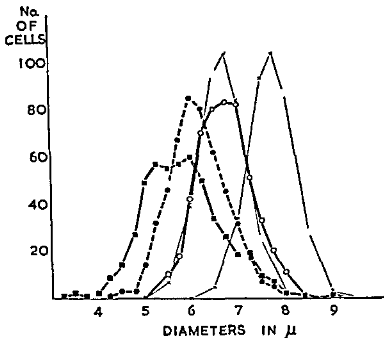


FIG 36 Erythrocyte diameter distribution curves (Price-Jones curves) made from the dried films of the peripheral blood of three patients with hereditary spherocytosis
o———o is a curve of a mild example of the disease ●– – – –● and ■═══■ are curves from typical cases of hereditary spherocytosis. The thin continuous lines indicate the maximum and minimum normal curves (Reproduced from *Practical Haematolo y* 2nd edn. p 60 Churchill London 19 6)

was 84 4 cu μ and the range 70 to 105 cu μ after splenectomy in 28 cases the mean was 85 9 cu μ and the range 62 to 99 cu μ (Table 5)

Spherocytosis can be appreciated if wet preparations of blood are examined (Fig 33) Gripwall (1938) and Dameshek (1939) have drawn attention to the unusual irregularity of the rouleaux which form in blood containing spherocytes the abnormal

Table 5

Hæmatological Data and the Effect of Splenectomy on Patients Suffering from Hereditary Spherocytosis

Pre or post splenectomy	Erythrocytes (minimum counts) (mill./cu. mm.)		Hæmoglobin (minimum values) (g./100 ml.)		MCV (cu. μ)		MCHC (%)		Reticulocytes (maximum counts) (%)		Serum bilirubin (mg./100 ml.)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Pre	2.0–4.9 (42)	3.57	7.3–16.8 (32)	11.4	70–105 (42)	84.4‡	32–40 (32)	36.4†	3–41 (48)	15.1	0.8–5.6 (28)	2.0
Post	4.0–6.0 (27)	4.92	12.6–17.8 (25)	14.9	62–99 (28)	85.9‡	30–40 (24)	35.5†	0.2–3.7* (30)	1.3	0.3–1.8 (18)	0.68

The figures in parenthesis indicate the number of patients investigated

* Three of the 30 patients had counts exceeding 2.5%

† The difference between these two means is significant ($t = 1.68$; $0.05 > P > 0.025$)

‡ The difference between these two means is not significant ($t = 0.72$; $P > 0.2$)

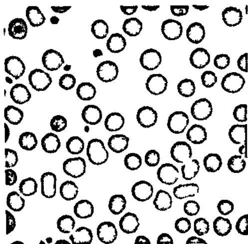


FIG 37 Photomicrograph of a blood film of a patient suffering from typical hereditary spherocytosis Female aged 1 1? The round contours and the deeper staining of the most spherocytic cells are well shown ?00

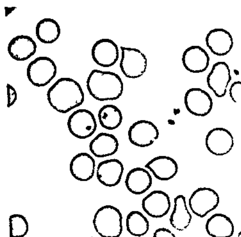


FIG 38 Photomicrograph of a blood film of a patient suffering from (?) atypical hereditary spherocytosis Female aged 3 Many of the erythrocytes contain irregularly shaped basophilic granules The Prussian blue reaction for iron negative Before splenectomy 1180

cells not fitting together as regularly and as tightly as do normal corpuscles

The hæmoglobin content of spherocytes is normal The hæmoglobin concentration is however slightly above the normal range (Table 5) (see later under *Chemistry of Spherocytes* p 104) In stained films spherocytes appear as small relatively deeply staining cells As a rule there is no trace of central pallor (Fig 37) Their diameters vary considerably the contours are usually conspicuously rounded Their size and staining is best appreciated when they are compared in the same field of a blood film with the larger less spherocytic reticulocytes which stain diffusely basophilic with the Romanowsky dyes

Punctate basophilia may be seen but this is not usually conspicuous in stained films Pappenheimer bodies and siderocytes are not normally present in appreciable numbers unless splenectomy has been carried out. Then they are continuously present but in very variable numbers (2–45% Douglas and Dacie 1953) The blood film reproduced as Fig 38 is exceptional in this patient, a girl aged 22 years in addition to diffuse punctate basophilia and small basophilic dots the size of Pappenheimer bodies about 5 % of the corpuscles contained much larger inclusions of irregular shape 1–2 μ in diameter which stained blue with Romanowsky dyes These larger inclusions did not seem to contain iron demonstrable by the Prussian blue reaction When the blood was examined 6 weeks after splenectomy the inclusions had disappeared

Reticulocytes In hereditary spherocytosis reticulocytes are normally present in far larger numbers than in health the count usually being between 5% and 20% The reticulocyte counts of the author s cases (before splenectomy) are shown in Table 5 Usually the reticulocyte counts remain at high levels throughout the patients lives unless splenectomy is carried out Occasionally however for reasons which are at present obscure erythropoiesis in the marrow may become greatly reduced or even completely suspended The peripheral reticulocyte count then falls to low levels and the patients may quickly go into an aplastic crisis (see p 110)

Erythroblastæmia Normoblasts are not commonly present in the peripheral blood of patients with hereditary spherocytosis Small numbers however may be found when the reticulocyte count is markedly raised particularly if the patient is seriously anæmic

Blood Picture at Birth

Microspherocytosis seems to be less conspicuous at birth than in later life (Hawksley 1936 Shapiro *et al* 1957) and as in hæmolytic disease of the newborn the hæmoglobin level and erythrocyte count

may be normal at birth (Hawksley 1936 Stamey and Diamond 1957 Shapiro *et al* 1957) only to fall possibly to dangerously low levels in the first few weeks of life The post natal fall in count is presumably associated with a failure of bone marrow regeneration rather than due to increased hæmolysis Further studies on this point are however required

Osmotic Fragility

Ever since the pioneer observations of Chauffard (1907) great interest has been taken in the phenomenon of the increased

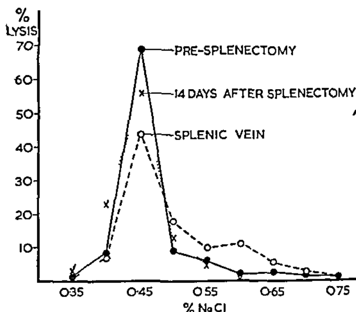


FIG 39 Increment hæmolysis osmotic fragility curves derived from tests carried out on a patient with hereditary spherocytosis before and after splenectomy There is evidence for a double population in the splenic vein blood with respect to osmotic fragility and to a lesser extent in the peripheral blood before splenectomy The curve is almost exactly symmetrical after splenectomy

osmotic fragility of the erythrocytes of hereditary spherocytosis Indeed at one time an increase in fragility almost came to be considered diagnostic of hereditary spherocytosis As already explained it is now recognized that this view was erroneous for the increased fragility depends upon spherocytosis which can be due to several causes

As discussed on p 37 the results of fragility tests have been generally reported either by recording the concentrations of saline which (*a*) cause just detectable lysis and (*b*) complete lysis or more completely by recording the percentage of hæmolysis caused by each saline concentration used The results of a quantitative test form a curve when plotted on graph paper Alternatively as referred to on p 39 increment hæmolysis curves can be drawn (Fig 39) These are particularly useful in studying patients with hereditary spherocytosis (see p 100)

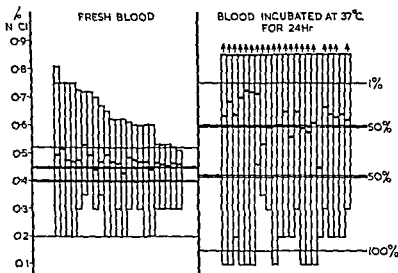


FIG 40 Results of osmotic fragility tests in 23 patients with hereditary spherocytosis *Left hand chart* results with fresh blood. *Right hand chart* results with blood incubated for 24 hours at 37 C

The saline concentrations causing 1% (top thin horizontal bar) 50% (thick horizontal bar) and 100% (bottom thin horizontal bar) lysis are recorded. † denotes > 1% lysis

The upper and lower thin horizontal lines represent the normal upper limit for initial (1%) hæmolysis and the normal lower limit for complete (100%) hæmolysis respectively

The two double horizontal lines represent the upper and lower limits for 50% hæmolysis (MCF)

All grades of increase in osmotic fragility are found in hereditary spherocytosis and in a small proportion of cases the curve falls close to the upper limit of the normal range Gansslen Zipperlen and Schuz (1925) reported that 10% of compensated cases had

a normal osmotic fragility and of the patients studied by Young (1955b) 25% *had osmotic fragilities within or near the normal* range The results of tests carried out on patients recently studied by the author are shown in Fig 40

Dacie (1943) reporting on the curves obtained with the blood from 24 patients suffering from hereditary spherocytosis[1] noted certain differences in the form of the curve in different patients The commonest type of curve was a tailed one with curves of this sort hæmolysis

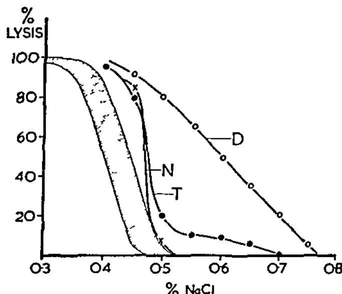


FIG 41 Different types of osmotic fragility curves in hereditary spherocytosis
N = normal type curve T = tailed curve D = diagonal curve
The shaded area represents the normal range

was first detected with saline of concentrations between 0 76% and 0 58 % *and only gradually increased in amount with diminishing* saline concentration until a point was reached at which 10–20% of the erythro cytes were lysed Beyond this point the curve became abruptly steeper and of approximately the same shape as the curve of a normal person The cell populations producing curves of this sort are clearly heterogeneous and include small proportions of unusually fragile cells In six patients diagonal curves were recorded here hæmolysis was first perceptible *with saline concentrations varying between* 0 80 % and

[1] It is probable in retrospect that one of these patients was suffering from acquired hæmolytic anæmia

0 68° and increased fairly steadily as the concentration of saline was reduced In five patients in whom the increase in fragility was slight the shape of the curve was normal or almost normal and there were only very small tails of fragile cells Further experience has confirmed the general truth of these observations but it is clear that curves intermediate in type between the extremes illustrated in Fig 41 occur It seems likely that a patient retains his own characteristic type of curve for long periods and that members of the same family more often than not have the same type of curve (Figs 42 and 44) There is however no close correlation between either the initial or the median fragilities

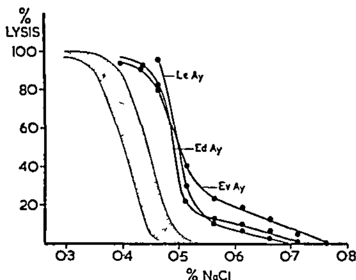


FIG 42 Osmotic fragility curves of three patients with hereditary spherocytosis belonging to the same family (brother sister and uncle) The shaded area represents the normal range

and the patients erythrocyte counts although it is true that in general those patients with the greatest increases in osmotic fragility are the most severely affected clinically

Vaughan (1937) associated flatness of the fragility curve with acute hæmolytic crises and severe anæmia This view received some support from the data published by Dacie (1943) which indicated that patients with normal type fragility curves tended to be less anæmic than those with diagonal curves the patients with tailed curves forming an intermediate group

The studies of Bolton (1949) Emerson (1954) and Emerson Shen Ham Fleming and Castle (1956) have gone a long way to explain the different types of curves which may be observed in hereditary

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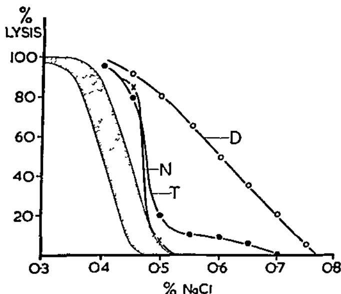


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was first detected with saline of concentrations between 0 76% and 0 58% and only gradually increased in amount with diminishing saline concentration until a point was reached at which 10–20% of the erythrocytes were lysed Beyond this point the curve became abruptly steeper and of approximately the same shape as the curve of a normal person The cell populations producing curves of this sort are clearly heterogeneous and include small proportions of unusually fragile cells In six patients diagonal curves were recorded here hæmolysis was first perceptible with saline concentrations varying between 0 80 / and

[1] It is probable in retrospect that one of these patients was suffering from acquired hæmolytic anæmia

subject did not undergo hæmolysis for 32 hours Dacie (1941) reported observations on ten patients Amounts of lysis varying from traces to 5% lysis were observed after 24 hours incubation and up to 50% lysis in 48 hours With normal blood only traces of lysis were to be seen after 24 hours and not more than 5% after 48 hours incubation It was also shown that in hereditary spherocytosis the abnormally rapid spontaneous lysis was a property of defective erythrocytes for lysis took place at an accelerated rate in saline suspensions of cells in the absence of plasma and as rapidly in normal plasma as in autogenous plasma lysis was somewhat slowed in plasma or serum which had been previously heated at 56 C for 30 minutes A general correlation was noted between rates of lysis and the degree of increase in osmotic fragility (before incubation) These observations have since been confirmed and extended by Caroli Etévé Paraf and Robineau (1949) Young Izzo and Platzer (1951) Young and Miller (1953) Selwyn and Dacie (1954) Selwyn (1955) Young and co-workers (1956) and Verloop and Bakker v Vardenne (1958) Selwyn and Dacie (1954) and Selwyn (1955) studied certain biochemical changes associated with the hæmolysis and also the effect of glucose upon it Several interesting facts were established The spherocytes of hereditary spherocytosis swell and take in sodium at the same rate as normal corpuscles they lose potassium at a normal or slightly accelerated rate These changes are markedly slowed in the presence of glucose as are the changes in normal corpuscles Lysis is not correlated with swelling of the corpuscles as suggested by Ham and Castle (1940a b) but rather with the rapidity of increase in osmotic fragility on incubation Selwyn and Dacie (1954) concluded tentatively that in hereditary spherocytosis lysis depended upon a defective cell membrane which underwent a degenerative irreversible shrinkage more rapidly than normal They also showed that significantly different patterns of autohæmolysis were observed with the erythrocytes from other hæmolytic disorders and that whereas excess glucose markedly retarded the hæmolysis of the hereditary spherocyte this was not necessarily true of the spherocytes of auto-immune hæmolytic anæmia or the rapidly hæmolysing erythrocytes of certain cases of hereditary non spherocytic hæmolytic anæmia (see p 189)

Data illustrating the effect of glucose on autohæmolysis in hereditary spherocytosis hereditary non spherocytic hæmolytic anæmia and normal subjects are shown in Fig 24 (p 44) In 21 patients with hereditary spherocytosis the range of hæmolysis

spherocytosis Both Bolton (1949) and Emerson and co workers (1954 1956) plotted their data as increment hæmolysis curves and showed that before splenectomy the distribution of sensitivity to osmotic hæmolysis of the erythrocytes in hereditary spherocytosis was often bimodal but became symmetrical and monophasic after splenectomy Emerson and co workers concluded that the tail of very fragile cells which may often be demonstrated is composed of cells escaping from the spleen after having been made more fragile therein (see also p 125)

Osmotic Fragility after Incubation for 24 Hours at 37 C ('Incubation Fragility) Emerson Shen and Castle (1946) Emerson Shen Ham and Castle (1947) Young (1947) Maier (1950) and Varadi (1951) observed that the increase in osmotic fragility resulting from incubation at 37° C was more marked in hereditary spherocytosis than in normal subjects Later Young Izzo and Platzer (1951) published details of their observations on 17 patients before splenectomy and concluded that the incubation test was particularly useful in detecting the slightest grades of abnormality In several instances the fragility of the blood suspected to be abnormal was significantly greater after incubation than that of normal controls whereas there had been no significant difference before incubation Curves illustrating the changes in a typical case are shown in Fig 23 (p 41) While there is no doubt as to the value and sensitivity of the incubation fragility' test in the author s view it has yet to be proved whether it will distinguish from normal the mildest examples of hereditary spherocytosis Even if it is admitted that the patient described by Dacie Mollison Richardson Selwyn and Shapiro (1953) whose erythrocytes reacted normally on incubation might not have been suffering from hereditary spherocytosis it has to be accepted that the incubation fragility test failed to distinguish from normal the blood of healthy parents of affected children considered by Young (1955a) and Ham (1955) on genetical grounds to be carriers of hereditary spherocytosis

The abnormally great increase in osmotic fragility which results from the incubation of the blood of patients with hereditary spherocytosis is associated with an increased rate of spontaneous hæmolysis (Selwyn and Dacie 1954 Young Izzo Altman and Swisher 1956) (see below)

Spontaneous Lysis on Incubation at 37° C (Autohæmolysis)

Ham and Castle (1940a b) reported that when blood from a patient with hæmolytic jaundice was incubated at 37°C hæmolysis occurred after 12 hours although blood from a normal

rate. Young and co-workers confirmed that hæmolysis was markedly slowed in the presence of glucose and found that mannose, adenosine, guanosine and inosine were also effective in retarding hæmolysis. Acidification of the blood with lactic acid to pH 6.8 also reduced hæmolysis but to a lesser extent than did glucose. In two patients the addition of glucose to blood actually accelerated hæmolysis *before* splenectomy; after splenectomy, from which the patients derived striking clinical benefit, glucose had as marked effect in reducing autohæmolysis as in typical cases of hereditary spherocytosis. The reason for these discrepancies is unknown. The present author has made a similar observation in three patients (see p. 109).

Although there is a wide variation between patients, there is a positive correlation between the rate of autohæmolysis and the osmotic fragility after 24 hours incubation (% lysis in 0.85% NaCl) ($r = 0.5300$, $0.02 > P > 0.01$ before splenectomy; $r = 0.811$, $P = 0.01$ after splenectomy (Fig. 43). Young and his colleagues (1956) reported a similar correlation and noted that the correlation between autohæmolysis and osmotic fragility was closer with incubated cells than with fresh cells.

Mechanical Fragility

Spherocytes were reported by Shen, Castle and Fleming (1944), Maier (1950) and Matthes (1950) to be unusually easily lysed by mechanical trauma. The most extensive data on this point are to be found in Young, Izzo and Platzer's (1951) paper. Young and his colleagues studied 18 patients (before splenectomy) and showed that the mechanical fragility of their erythrocytes was on the average 4–5 times that of normal controls; after incubation the average mechanical fragility of the patients' corpuscles was about 8 times that of the controls. Young (1955b) pointed out that the normal or almost normal survival of hereditary spherocytes after splenectomy (see p. 121) suggests that abnormal mechanical fragility *per se* does not significantly shorten erythrocyte life span *in vivo*.

Serology

Boorman, Dodd and Loutit (1946) and Loutit and Mollison (1946) reported that the direct antiglobulin (Coombs) test was negative in patients with hereditary spherocytosis. The observations of most later workers including those of the author have confirmed this. Young, Izzo and Platzer (1951), for example, reported negative results in 28 patients. Positive reactions were, however, reported by Singer and Motulsky (1949), Wright, Dodd and Bouroncle (1949) and Wright, Dodd, Bouroncle, Dean and Zollinger (1951). It is possible that some of these positive reactions have been due to the use of an unsuitable technique. However, in a few patients in hæmolytic crises it seems probable

at 48 hours without additional glucose was 7 5–47 5%[1] (mean = 28 5%) with glucose the range was 0 9–15 5% (mean=6 7% 18 patients) The results expected in health are 0 4–4 5% (without glucose) and 0 03–0 4% (with glucose)

Young Izzo Altman and Swisher (1956 1958) have recently published further extensive observations on autohæmolysis in hereditary sphero cytosis In every case including 14 patients whose blood showed minimal spherocytosis and normal or almost normal osmotic fragility (before incubation) autohæmolysis proceeded at an abnormally fast

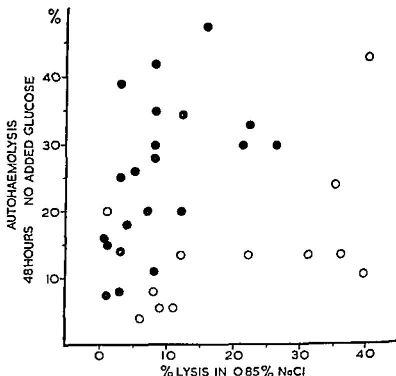


FIG 43 Correlation between autohæmolysis (% hæmolysis after 48 hours incubation at 37 C) and osmotic fragility after 24 hours incubation at 37 C (% lysis in 0 85% NaCl)
● = pre splenectomy (21 patients) ○ = post splenectomy (12 patients)

[1] These data do not include observations made on two atypical (? non hereditary) cases referred to on p 109 and on a patient with hereditary spherocytosis plus the nephrotic syndrome and amyloid disease

suggested a definite deficiency in the lipid content of hereditary sphero cytes Crosby (1952) quoting the work of Erickson and co-workers correlated this lipid deficiency with the reduced surface area of the spherocytes He suggested that in the maturation of the hereditary spherocyte there was a disproportionately great loss of surface area and presumably of the materials that comprised the cellular surface

More recent work on the chemistry and metabolism of the hereditary spherocyte is considered under *Pathogenesis of Hereditary Spherocytosis* (p 133)

Hæmoglobin The hæmoglobin in hereditary spherocytosis is generally considered to be of the normal adult variety (Hb A) only except in early infancy when the normal proportions of fœtal hæmoglobin (Hb F) are present Using a sensitive physico chemical method (combined alkali denaturation and ultraviolet spectrography (Beaven Ellis and White 1956)) White Beaven and Ellis (unpublished observations) failed to demonstrate Hb F in nine patients with hereditary spherocytosis (children and adults) and eleven members of their families

Recently however an abnormality in the hæmoglobin in hereditary spherocytosis has been reported by Breuer de Vries Eckel and Matoth (1958) In moving boundary electrophoresis raising the temperature or lowering the ionic strength was found to cause two components of hæmoglobin to separate in the descending limb whereas with hæmoglobin from normal subjects only one component separated The phenomenon was noted in hereditary elliptocytosis and atypical congenital hæmolytic anæmia as well as in hereditary spherocytosis Breuer and his co workers suggested that it indicated a weakening of the forces which bound together the protein sub units of the hæmoglobin molecule and that these forces might be concerned with the maintenance of the integrity of the erythrocyte

Bile Pigment Metabolism in Hereditary Spherocytosis

The usual range of plasma bilirubin levels in hereditary spherocytosis has been referred to on p 89 Urobilinogen excretion in the fæces is characteristically increased and may be many times the normal (Goldschmidt Pepper and Pearce 1915 Watson 1937 Barker 1938 Crosby and Akeroyd 1952)

Watson s (1937) data are the most extensive Ten of his patients suffered from hereditary spherocytosis their excretion of urobilinogen varied from 136 to 2 475 mg per day the average was approximately 900 mg (about six times normal) Barker (1938) studied three patients their daily excretion ranged from 500 to 1 087 mg Watson made the point that the excretion of urobilinogen in the urine is only slightly to moderately raised in uncomplicated cases in his patients the total daily excretion

that antibody development leading to auto immunization may have been superimposed upon the original congenital disease (Dameshek and Bloom Case 6 1948 Mendes de Leon 1952 Young and Miller 1953 Michel Bornemann and Thomas 1955)

Mendes de Leon (1952) and van Loghem Mendes de Leon and van der Hart (1955) in studies on the effect of incubation on normal corpuscles suspended in patients sera reported that in many cases of hereditary spherocytosis (50 % of 22 cases) and in other types of hæmolytic anæmia a hæmolytic serum factor was present After splenectomy the hæmolytic activity of the serum was normal in eight out of nine patients The significance of these observations has not yet been elucidated

Erythrocyte Chemistry in Hereditary Spherocytosis

The few chemical observations available point to differences between hereditary spherocytes and normal corpuscles

Maizels (1936) concluded that the potassium and water concentrations of the erythrocytes were low in hereditary spherocytosis the sodium concentrations were normal and the hæmoglobin concentration increased He pointed out that the hæmoglobin and water concentrations would have an inverse relationship and that the total cation concentrations would be dependent on the water concentration He also pointed out that as a small proportion of the cell water is bound by the hæmoglobin the high hæmoglobin concentration in spherocytes would result in a higher proportion of the cell water being bound Maizels studied three patients before and after splenectomy the hæmoglobin concentration in the erythrocytes fell by about 10% after operation and their water content increased slightly the potassium concentrations and cell volumes rose slightly

Erickson Williams Hummel Lee and Macy (1937) studied the erythrocytes of three children with hereditary spherocytosis (two after splenectomy) in one patient before splenectomy they found the cell potassium concentration to be high in the other two children examined after splenectomy the concentrations were normal These observations are at variance with those of Maizels (see also below)

Selwyn and Dacie s (1954) work was based on ten patients and has confirmed Maizels s observations They found the hæmoglobin concentration to be high mean 37 g range 34–40 g per 100 ml cells (normal range 32–36 g per 100 ml cells) the cell water to be low range 65–69% (normal range 69–72%) the cell sodium to be normal range 8–12 m equiv /litre cells (normal range 8–12 m-equiv /litre cells) and the cell potassium to be low range 75–95 m equiv /litre cells (normal range 100–114 m equiv /litre cells) After splenectomy there was a shift towards the normal (The effect of incubation on the chemistry of spherocytes has already been referred to (p 101))

Erickson and co workers (1937) also studied the corpuscular lipids in certain cases of hæmolytic anæmia In four patients with hereditary spherocytosis the total lipid averaged 359 $\times 10^{-11}$ mg per cell and in three patients after splenectomy 330 $\times 10^{-11}$ mg per cell These figures

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ranged from 1 to 10 mg compared *with* the normal excretion of 0 6 mg When urobilinuria of marked degree occurred this was in Watson s view, nearly always due to complications such as infection severe anæmia infarction the effects of anæsthesia or ' *hæmolytic crisis —all of which affected the function of the* liver In one jaundiced patient with marked urobilinuria however none of these factors was apparently operating and it appeared likely that liver function was concurrently disturbed at least in respect of *its power of excreting bile pigment*

Erythrocyte Life Span in Hereditary Spherocytosis

Dacie and Mollison (1943) carried out the first erythrocyte survival experiments in hereditary spherocytosis Using the Ashby method they found that the corpuscles of one patient were completely eliminated from the circulation of a healthy recipient within 14 days When the experiment was repeated one year after the patient had undergone splenectomy only 32% of the patient s corpuscles were present 8 days after the transfusion elimination being complete in 19 days

The recent introduction of the ^{51}Cr method has permitted measurement of the life span of the erythrocytes in the patient s own circulation which was not possible using the Ashby technique So far the published data are not extensive Read Wilson and Gardner (1954) reported ^{51}Cr half times of 11 12 14 and 18 days (normal range 23–30 days) while Motulsky Giblett Coleman Gabrio and Finch (1955) indicated that the rate of destruction was usually 6–8 times normal Schloesser Korst Clatanoff and Schilling (1957) have published data on four patients the pre splenectomy ^{51}Cr half times were 10 11 11 and 17 days respectively (normal 30–42 days) and in one patient after *splenectomy 29* days Hughes Jones and Szur (1957) in three clinically mild cases reported mean cell life spans of 18 19 and 27 days respectively Other patients more recently studied in the author s laboratory *had mean cell life spans of approximately 8 12 14 and 19 days* (Lewis Szur and Dacie (unpublished observations))

Schrumpf (1951) in a classic experiment and Emerson and co workers (1956) have shown that if hereditary spherocytes are transfused to a healthy recipient lacking a spleen their survival is virtually normal The curve published by Emerson and his co workers is however definitely curvilinear and although a small proportion of cells survived at least 120 days 50% appear to have been eliminated in about 40 days

Schrumpf (1956a) in further experiments claimed that hereditary

spherocytes were eliminated from the circulation faster by a recipient who had hereditary spherocytosis (with an intact spleen) than by a normal subject After the recipient with hereditary spherocytosis had been splenectomized the survival of transfused hereditary spherocytes was found to be normal Jandl Greenberg Yonemoto and Castle (1956) and Schloesser and co workers (1957) have however made the contrary observation namely that the erythrocytes from a patient with hereditary spherocytosis survive better when transfused to another patient with hereditary spherocytosis than in a normal subject This could be interpreted as being due to the patient s spleen already engorged with blood being less able to filter off the transfused spherocytes than a normal spleen The role of the spleen is considered in more detail on pp 134–138

VARIANTS OF HEREDITARY SPHEROCYTOSIS

Mild Forms of the Disease Gansslen Zipperlen and Schuz (1925) and Campbell and Warner (1925–26) were among the first writers to stress the existence of very mild forms of the disease As a result of studies involving about 120 patients Gansslen and co workers described three main types of the disease a *complete form* a *compensated form*—35% of the patients without anæmia 5% with polycythæmia 40% without jaundice 30% without splenomegaly 10% without increased osmotic fragility and a *mild form* which they referred to as the leichte hamolytische Konstitution In this last group were placed healthy people with perhaps slightly increased degrees of anisocytosis of the erythrocytes minor fragility changes slight and inconstant hyperbilirubinæmia but with no anæmia or splenomegaly Out of 68 members of a family comprising 161 persons in three generations Gansslen and his colleagues found ten completely healthy subjects eleven with the complete disease thirty four with the compensated disease and thirteen with the mild carrier form

Subsequent writers have tended to overlook the possibility of the extremely mild forms described by Gansslen although compensated cases have been well recognized On the other hand the diagnosis of leichte hamolytische Konstitution should not be made unless repeated careful studies have been carried out particularly as slightly increased anisocytosis is of frequent occurrence and small fragility changes are difficult to be certain about The author now feels if the osmotic fragility of the fresh blood of a patient suspected of having hereditary spherocytosis is

strictly normal on repeated testing that the alternative diagnosis of hereditary non spherocytic hæmolytic anæmia is more likely to be correct He has not yet seen a patient of this type where the presence of the disease in an unmistakable form in another member of the family made the diagnosis of hereditary spherocytosis (with normal osmotic fragility) virtually certain (see below)

Of 28 patients investigated before splenectomy by the author in recent years six had minimum hæmoglobin values exceeding 13 5 g per 100 ml in three the minimum value was greater than 14 8 g per 100 ml Six of the 28 patients had erythrocyte osmotic fragilities (before incubation) just outside the normal range (Fig 40[1]) In two patients (Cases 8 and 9 of Dacie and co workers 1953) the results were entirely normal As it is debatable whether these two patients should be accepted as cases of hereditary spherocytosis they have been excluded from consideration and are with other atypical cases discussed in Chapter 4 (p 193) There is no close correlation between hæmoglobin levels and the extent of the increase in osmotic fragility—which may be very definitely abnormal despite a normal hæmoglobin However as mentioned earlier *the most abnormal fragility curves are usually associated with active and incompletely compensated hæmolysis*

The usefulness as diagnostic aids of studying the rate of autohæmolysis and the osmotic fragility after 24 hours incubation at 37 C has already been referred to (p 100) Young (1955a) mentioned for instance seven patients from three families with normal or almost normal osmotic fragility before incubation in whom the test gave definitely abnormal results after the blood had been incubated In the author s experience however the measurement of the osmotic fragility of incubated blood has been of more value in excluding hereditary spherocytosis as the diagnosis in a mild case of hæmolytic anæmia than in confirming the diagnosis

There is some evidence which suggests that mildness of the disease may be a family characteristic For instance families have been observed in which anæmia has been slight and the increases in osmotic fragility have been similar and minimal in all of the affected members the fragility curves being normal or almost normal in shape (Wiedemann 1942 Discombe 1948 Young Izzo and Platzer 1951) It is not clear whether these cases represent a slightly atypical genetically different type of hereditary spherocytosis or whether the uniform mildness of the *disease is due to the influence of the unaffected parent on the* expressivity of the gene The author described in the first edition of this book (Dacie 1954 p 66) a family of this type in which four boys were affected with a mild form of the disease (Fig 44)

[1] The results in five patients are not recorded in the figure as no observations were made after incubating the blood for 24 hours at 37 C.

Atypical Cases With Spherocytosis As already mentioned (p 85) cases of apparent hereditary spherocytosis in which family studies give absolutely normal results are not rare Usually the hæmatological and clinical findings are typical and a diagnosis of hereditary spherocytosis can be made with confidence However a small number of patients have been observed with both parents apparently normal who show abnormal hæmatological features (or react abnormally to splenectomy)

Young and his colleagues have described two groups of this type Young (1955a) mentioned three patients whose blood

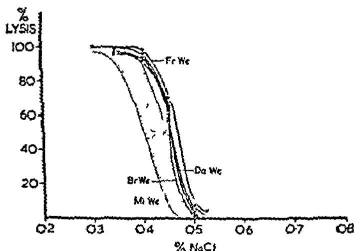


FIG 44 Osmotic fragility curves of four brothers all suffering from hereditary spherocytosis (Cases 1–4 of Dacie 1954) The shaded area represents the normal range

picture although thought to be typical of hereditary spherocytosis before splenectomy became entirely normal subsequently It seems improbable that such cases are variants of hereditary spherocytosis Later Young and his co workers (1956) described two patients under the title atypical (Group B) spherocytosis whose disorder differed from the typical hereditary type in that the rate of autohæmolysis of the patients erythrocytes was not reduced by the addition of glucose Curiously enough after splenectomy the patients corpuscles behaved normally in this respect The present author has made similar observations in two children of different families whose parents also were apparently

normal as well as in an adult (with a positive family history) who had the nephrotic syndrome and amyloid disease in addition to hereditary spherocytosis The significance of these observations is not yet known They suggest that the biochemical abnormality in hereditary spherocytosis may not be uniform and support the concept that more than one type of the disorder (with sphero cytosis) exists (see also p 133)

Hereditary Spherocytosis Combined with other Inherited Traits

Patients with hereditary elliptocytosis whose blood in addition shows well marked spherocytosis have been observed As will be discussed on p 164 there is reason to believe in most instances at least that this blood picture can be produced by hereditary elliptocytosis alone The *presence of hereditary elliptocytosis and hereditary spherocytosis in* the same family and the consequent production of undoubted double heterozygotes does not seem yet to have been reported Examples of hereditary spherocytosis combined with sickle cell trait have however been described Both the patients mentioned by Smith and Conley (1954) and de Torregrosa Ortiz and Vargas (1956) responded well to splenectomy

Jonsson and Zetterstrom (1954) and Zetterstrom and Strindberg (1958) have described unusual cases of hæmolytic anæmia with sphero cytosis in infancy which may possibly be variants of hereditary spherocytosis Zetterstrom and Strindberg s two infants also had thrombocytopenia of the amegakaryocytic variety and skeletal or renal abnormalities

The combination of hereditary spherocytosis with pernicious anæmia is suggested by the report of Ercoli (1954) Eight patients were studied in two generations Three of them had megaloblasts in their bone marrow six had raised reticulocyte counts and the osmotic fragility was reported to be increased Price Jones curves showed both micro cytes and macrocytes Although the presence of neither disorder was proved the possibility of the remarkable combination cannot be excluded

Aplastic (Anæmic) Crises in Hereditary Spherocytosis

The occurrence of crises in the course of hereditary sphero cytosis has been known for many years (Tileston 1922 Dawson 1931 Dameshek and Bloom 1948) They were described as crises de déglobulization by French writers in the early years of this century More recently it has been recognized that crises may affect several members of the same family concurrently or successively and that in most instances an obvious increase in hæmolysis with deepening jaundice is not the cause of the crisis

Examples of epidemic familial crises have been described by Murray Lyon (1935) Scott (1935) Dedichen (1937) Dameshek

(1941) Lyngar (1942) Horne Lederer Kirkpatrick and Leys (1945) Li Voth and Osgood (1950) Marson Meynell and Tabbush (1950) Battle (1952) Ingham (1952) Margolis (1953) Haase (1954) Esmond Quinn and Peters (1955) Betke Debatin and Sauthoff (1955) Moorhouse and Mathewson (1956) Denny Bird and DuVal (1958) and Greig Metz Bradlow Theron and Morris (1958) It is noteworthy that Murray Lyon Scott Dameshek and Lyngar all remarked on the low reticulocyte counts of their patients at the height of their crises when they were most anæmic Scott (1935) considered that erythropoiesis was failing to keep pace with destruction and Dameshek (1941) suggested that the crisis might develop not only because of increased hæmolysis but also because of inhibition of the bone marrow due to unusual splenic activity Dameshek also remarked on the leucopenia that was present at the time of the crises and suggested that this might be due to a splenic influence also Lyngar (1942) concluded that the crisis in the patients he studied was due to bone marrow failure rather than to increased hæmolysis The fact that failure of the bone marrow could lead to severe anæmia in a patient with hæmolytic anæmia had however been appreciated earlier than this

Banti (1913b) made a distinction between hæmopoietic and anhæmopoietic types of hæmolytic anæmia It seems probable however that the patient he described as having anemia emolitica splenomegalica anemopoietica had in reality thalassæmia minor (of the Rietti Greppi Micheli variety)

Dawson (1931) without question dealt with hereditary spherocytosis He did not refer to marrow aplasia but he was well aware of the importance of the marrow in relation to how anæmic a patient became He reported that one (elderly) subject with cholangitis had an erythrocyte count of 3 05 millions per cu mm but only 0 6 reticulocytes he remarked that this showed that there was but little fight left on the part of the marrow

Pepper and Wise (1933) gave a detailed account of a boy of 18 years almost certainly suffering from hereditary spherocytosis who became febrile and severely anæmic Only very occasional reticulocytes were present in the peripheral blood and he was far less yellow than in former attacks Pepper and Wise attributed the anæmia and reticulocytopenia to bone marrow failure and discussed whether the attack was associated with an intercurrent infection The bone marrow itself was unfortunately not studied

Detailed studies on the genesis of crises in hereditary spherocytosis have more recently been published by Owren (1948) Dameshek and Bloom (1948) and Gasser (1950 1951) and it is now generally agreed that crises are almost always brought about by marrow aplasia

Owren described the course of the crisis in six patients four of them were members of the same family all became ill within a few days The other two patients belonged to different families In each case the patient suddenly developed pyrexia two patients had rigors one of them experienced abdominal pain with vomiting The pyrexia lasted about 10 days the patients temperature returning to normal at about the same time as the reticulocytes reappeared in the peripheral blood The patients jaundice was observed to decrease as they became more anæmic whilst the size of their spleens remained unaltered In all his patients Owren found a severe reticulocytopenia at the height of their crises—the counts varied from 0 to 0 3% In addition there was granulocytopenia the total neutrophil counts ranging from 760 to 2 400 per cu mm and thrombocytopenia with counts ranging from 30 000 to 160 000 per cu mm Bone marrow studies showed that the peripheral pancytopenia was a reflection of an acute hypoplasia of the bone marrow particularly affecting erythropoiesis

Dameshek and Bloom (1948) studied seven patients three of them having been reported previously by Dameshek (1941) in these patients the crisis was interpreted as being due to the combination of a marked exacerbation of the hæmolytic mechanism with an arrest of maturation of the developing erythroblasts in the marrow The marrow inhibition was attributed to a pathologically hyperactive spleen Reticulocytopenia was marked in six of the patients all suffering from major crises and there were lesser degrees of granulocytopenia and thrombocytopenia In one patient (Case 7) reticulocytes were reported to be absent from the peripheral blood for at least 5 days Marked spherocytosis was a feature of the crises in all Dameshek and Bloom s patients Serial bone marrow studies were carried out on one patient (Case 7) From this it appeared that at the height of the reticulocytopenia in the peripheral blood there was a maturation arrest affecting erythropoiesis It is possible though that the appearances were those of early recovery of marrow function and that a puncture done earlier in the crisis would have shown marrow hypoplasia The exaggerated spherocytosis in the peripheral blood can be explained as the direct consequence of diminished formation as the circulating cell population would become increasingly older and more spherocytic as time passed

Gasser (1950 1951) described the occurrence in children of aplastische Erythroblastenkrisen (akute Erythroblastopenie) in the course of various illnesses including hereditary spherocytosis The sequence of events is well illustrated in his 1950 paper Like Owren he noted the disappearance of reticulocytes and erythroblasts in the bone marrow the marked reticulocytopenia in the peripheral blood and the rapid onset of anæmia He also recorded an increase in osmotic fragility and a fall in serum bilirubin as the anæmia progressed

Later studies *e g* those of Emery and Lemmon (1954) White (1956) and Greig and co workers (1958) have confirmed the correctness of the conception *of marrow aplasia The aregenerative phase lasts as a rule* from 7 to 10 days or even 14 days Leucopenia and thrombocytopenia as observed by Owren (1948) Dameshek and Bloom (1948) and Sansone (1955) do not always accompany the reticulocytopenia and the pattern of response may vary even in the same family For instance of the two children reported by Haase (1954) who developed reticulocytopenia after

a grippal infection one had 3 000 and the other 40 000 leucocytes per cu mm Similarly one of the patients referred to by Esmond Quinn and Peters (1955) who had a reticulocyte count of only 1 4 % had 46 000 leucocytes per cu mm Sansone (1955) noted in his patient who had leucopenia and thrombocytopenia as well as reticulocytopenia that rises in the granulocyte and platelet counts preceded the rise in reticulocyte count

The cause or causes of an aplastic crisis have not been established The fact that several members of a family may be affected simultaneously or successively suggests strongly that infections may be precipitating factors Whether the depression of the bone marrow results from the direct action of a virus or toxin or is brought about indirectly by some as yet unknown mechanism is uncertain It certainly seems that compensatory erythropoiesis in hereditary spherocytosis is delicately poised Gasser s observations suggest that a temporary depression of erythropoiesis is not uncommon in children as a result of infections and intoxications however it is only in hæmolytic anæmia that the results are serious

The role of increased hæmolysis in bringing about crises needs reconsideration in the light of the work referred to above Clearly excessive hæmolysis alone is much less important than was at one time thought and this is possibly true of minor crises also For instance it is interesting to note that the two patients described by Denny Bird and DuVal (1958) had both suffered from repeated but less severe episodes of weakness chills fever abdominal pain nausea and vomiting which in a more severe form were shown to be the accompaniments of aplastic crises

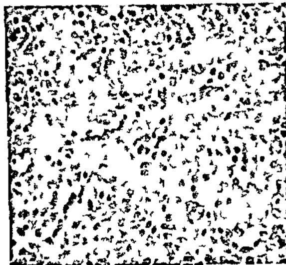
PATHOLOGY

Bone Marrow The bone marrow is characteristically hyperplastic fat cells are absent partially or completely from the marrow of the flat bones (Fig 14 p 27) and red marrow is found in areas in the long bones normally fatty In children hyperplasia of the marrow sometimes leads to widening of the diploe of the skull and radiological appearances rather similar to those seen in severe Cooley s anæmia (Caffey 1937) The marrow hyperplasia is due to proliferation of the normoblasts in severely anæmic patients they may be the predominant marrow cell Morphologically erythropoiesis is essentially normoblastic in type the developing cells being normal in size Mitotic figures are increased in number Megaloblastic change is extremely rare

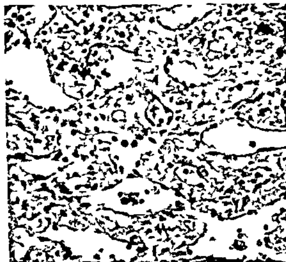
but has been observed for example by Davidson (1952) in a pregnant patient and in an elderly subject by Matthews (1954 unpublished observations) In both the above instances the megaloblastic change was the result of deficiency of folic acid not of B_{12} (see also p 29) It seems quite clear that normally in the otherwise healthy subject erythropoiesis can be maintained at many times the normal rate for many years without any deficiency of essential hæmopoietic factors developing

Extramedullary erythropoiesis has been observed from time to time Paravertebral masses containing bone marrow were described by Dawson (1931) Bamatter (1932) and Hartfall and Stewart (1933) *masses in the costovertebral angles by Gleave* (1936) and paravertebral subpleural nodules by Turnbull (1936) The incidence of intrathoracic bone marrow tumours in hæmolytic anæmia including hereditary spherocytosis has recently been reviewed by Paraf Decroix and Caroli (1957) who describe a further example in a patient with a chronic hæmolytic anæmia of doubtful type

Spleen The spleen is always enlarged but the enlargement is rarely extreme The largest spleens are found in the most severely affected patients After excision the spleen of an adult patient is usually found to weigh between 500 and 2 000 g *According to Wiland and Smith (1956) the spleen averages five* times the normal weight for the patient s age Infarcts are not usually found and adhesions if present are rarely extensive The vessels at the splenic hilum are not conspicuously large On section the spleen is characteristically a dark plum colour firm to the touch and looks as if it were deeply congested with blood the vessels and fibrous trabeculæ and Malpighian bodies are not usually conspicuous Microscopically the appearances are characteristic (Vaquez and Aubertin 1908 Guizzetti 1912 Eppinger 1920 Meulengracht 1922 Thompson 1932 Turnbull 1936 Klemperer 1938 Wiland and Smith 1956) The Malpighian bodies are usually normal in size but are widely separated by a pulp filled with blood Most of the erythrocytes are packed in the pulp cords in contrast the sinuses are often empty and may be lined by conspicuous almost cuboidal endothelial cells (Fig 45) There *is usually no increase in collagen although there may* be a slight increase in argyrophil reticulin fibres Macrophages containing *erythrocytes are not easily found but there is usually a moderate* amount of hæmosiderin present in phagocytic cells or as a diffuse impregnation Siderotic granules are rare in childhood but are frequently seen in older subjects (Wiland and Smith 1956)



a



b

FIG 4 ( ) Section of plecn remo l at operation f om a p at ent with l reditary p l rocytosi Tl enou nuse are mpty an l con picuou the pulp i o rf llc l witl rythrocyt H and E × 60

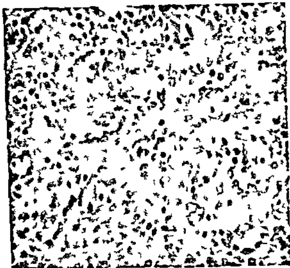
(*b*) S ction of spleen rem v l t ope ti n f om a pat ent with l edit ry spherocyto Tl e pl en h s be n p rfused fo hour w th saline at *100 mm Hg* pres ure via tl e splen c art ry Th pulp st ll contain many erytl ocytes (see D ic 1943) H a d l × 360

T f *p* 114

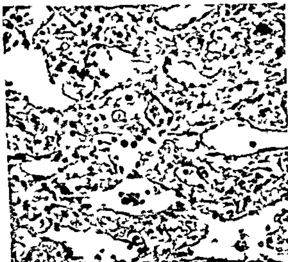
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a



b

F c 4 (a) Secti n of ;l n rem ve l at operat on from a pati nt with l ered tary spl ero vto Tl ven u ses a e e npty and consp uous the pulp i o erfill d vitl ervtl rocvtes H and E × 360

(b) Section of spleen remov d at operation f om p t nt witl he ed tary spherocyto Tl e pleen ha be perfused for ho ir witl sal ne at 100 mm Hg pres u tl e spl n c rt ry The pulp till conta n many erythrocyt (see Dac 1943) H and E × 360

T f p 114

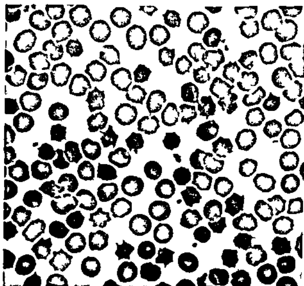


Fig 46 Photomicrograph of a blood film of a patient with hereditary spherocytosis Male aged 4 Splenectomy 11 years previously Hb 1[illegible] 8 g per 100 ml 1 [illegible] reticulocytes
Note the abnormal degree of crenation which is commonly seen in post splenectomy films × [illegible]00

According to Wiland and Smith foci of erythropoiesis are commonly found but this has not been the present author s experience

The exact location in life of the erythrocytes in the spleen of hereditary spherocytosis has been a subject of controversy The relatively empty sinuses seen in histological preparations of spleens removed at operation are presumably filled with blood in life The question is whether or to what degree the extreme congestion confined to the pulp is an artefact Knisely (1936) claimed as a result of direct inspection of the spleens of anæsthetized small animals that all the blood was contained in life in the sinuses and that its presence in the pulp in fixed sections was due to agonal changes Knisely s conclusions were disputed by MacKenzie Whipple and Wintersteiner (1940) using a similar technique However Gripwall (1938) in reporting a study on patients with hereditary spherocytosis claimed that sections of small pieces of two spleens removed at the time of operation and rapidly fixed showed the sinuses to be well filled with blood and not compressed by an overloaded pulp (see also p 135 under *Pathogenesis*)

The prevailing view at the present time seems to be that the great bulk of the blood is in fact in the pulp cords of the spleen during life

Other Organs The changes in other organs as revealed by studies on fatal cases are less characteristic There is often a moderate amount of hæmosiderosis of the liver but myeloid metaplasia is seldom seen Giant cell hepatitis has been observed in a fatal case in infancy (Bain Wang and Misanik 1957) The frequency of pigment gallstones has already been mentioned There is a variable amount of iron in the kidney (Turnbull 1936) The microscopical appearances of sections of a crural ulcer were described by Turnbull (1936) as those of chronic inflammation

Beinhauer and Gruhn (1957) reported on a skin biopsy of an erythematous (? pre ulcerative) area as showing hyalinization of collagen fibres in the dermis endarteritis and focal aggregates of histiocytes macrophages and lymphocytes

DIFFERENTIAL DIAGNOSIS

The most important diagnostic features of hereditary spherocytosis are as follows it is a congenital hæmolytic disease inherited as a Mendelian dominant the gene for which has a very variable penetrance the fundamental abnormality resides in the erythrocytes spherocytosis and increased osmotic fragility are characteristic although not diagnostic normal erythrocytes survive well after transfusion the results of splenectomy are excellent (see later) No disease presents a picture quite like this

and when all the signs are present diagnosis is simple Difficulty arises in the mild or atypical case for instance when the disease appears not to be congenital when the family history is negative when the osmotic fragility is normal and when the morphology of the erythrocytes is unusual or when the patient is seen for the first time in an aplastic crisis Most of these points have already been dealt with The fact that the patient is an elderly subject should not be allowed to weigh too heavily against the diagnosis of hereditary spherocytosis examination of relatives may reveal the disease in an unmistakable form Nor should the absence of a positive family history be deemed too important in an obviously congenital case if the rest of the picture is typical As mentioned on page 85 in some instances it seems probable that the disease may exist in a relative in such a mild form that its certain recognition is impossible by present methods alternatively, there is the less likely possibility of a new mutation

It is undoubtedly true that occasionally the erythrocyte osmotic fragility may fall almost possibly even quite within the normal range This may lead to confusion between two distinct diseases mild hereditary spherocytosis and hereditary non spherocytic hæmolytic anæmia if too much importance is given to the results of the fragility test alone In these cases it is important to investigate as many relatives of the patient as possible Fortunately study of the effects of incubation at 37° C on osmotic fragility and of the rate of autohæmolysis may also help greatly in differentiation (see p 189)

TREATMENT OF HEREDITARY SPHEROCYTOSIS

Splenectomy

The late results of splenectomy in hereditary spherocytosis are almost uniformly excellent Gansslen (1922) reported that nine out of ten patients were clinically cured—the one failure was a patient who died of a post operative portal vein thrombosis Thompson (1936) reported uniform and permanent relief in 18 patients and Cowan (1936) the same good results in 20 patients More recently Welch and Dameshek (1950) reported that every one of their 38 patients experienced complete clinical remissions Edwards (1951) obtained excellent results in 24 out of 25 patients —the one failure is discussed later (p 118) and Young Izzo and Platzer (1951) reported complete remissions in 16 patients Twenty eight patients studied by the author have undergone splenectomy all have done well

According to Dawson (1931) the first successful splenectomy in hereditary spherocytosis was carried out unwittingly by Spencer Wells in 1887 His patient was a woman aged 27 who had had attacks of jaundice since 9 years of age She had an abdominal tumour which was thought to be a fibroid this however turned out to be a very large spleen. Dawson reported that the osmotic fragility of her erythrocytes was still increased when he examined her blood about 40 years later Clinically she was then in good health Her son underwent cholecystec tomy and splenectomy at the age of 14 his erythrocytes also were reported by Dawson to be fragile

Splenectomy does not seem to have been performed again with benefit to the patient until Micheli s (1911) success in an acquired case stimulated other operators to carry out splenectomy in hæmolytic anæmias In England at a discussion at the Royal Society of Medicine early in 1913 several successful operations were referred to (Wynter 1912–13) By 1922 Tileston was able to write in the congenital type of hemolytic jaundice a permanent cure may be predicted as the result of splenectomy

Indications for Splenectomy It is now generally agreed that the spleen should be removed from any patient suffering from typical hereditary spherocytosis who is continuously anæmic or has a clinical degree of jaundice or who gives a history of an aplastic crisis The results of the operation are so good and the direct operative mortality nowadays so low that the operation should be carried out in all patients except in the completely compensated and sympton free cases The spleen has been removed successfully in infancy (Conrad and Schmidt 1946) even in the neonatal period (to avert kernicterus) (Roddy 1954) this should certainly be done if anæmia is so severe that repeated transfusions are essential The operation should however be postponed to later in childhood if possible Glenn Cornell Smith and Schulman (1954) recommended 3–4 years as the most suitable age for splenectomy and Young (1955b) 4–5 years Splenectomy in very young children should certainly be avoided if at all possible for there is a growing realization that infants whose spleens have been removed are probably more susceptible to infections than older children and adults (see p 129) However the possibility of post operative infections should not in the author s view be taken to forbid splenectomy too strongly in the first year of life if the degree of hæmolysis is such that repeated transfusions are required

Leaving aside the problem of an increased risk of infections after splenectomy—which may possibly apply also to older children and adults to a small degree there are good reasons for carrying out splenectomy in later childhood for the longer hæmolysis is allowed to continue the greater are the chances of the occurrence

of potentially serious complications such as aplastic crises or gallstone formation to say nothing of the possibility of retardation of growth and general vague ill health as the result of chronic anæmia (see p 88) If gallstones are present they should be removed at the time of splenectomy it at all possible

Splenectomy has been carried out at the time of an aplastic crisis (Denny Bird and DuVal 1958 Greig *et al* 1958) There is no evidence however that the operation accelerates recovery Of the two patients of Denny Bird and DuVal one was splenectomized the other was not Splenectomy was carried out on the 6th day of the crisis and was immediately followed by a fall in serum bilirubin The reticulocyte count did not however start to rise until 5 days later The pattern of the disease was the same in both patients and did not seem to be influenced by splenectomy The patient of Greig and his co workers was splenectomized on the 11th day of the crisis There was a prompt rise in leucocyte and platelet counts but the reticulocyte count did not rise until the 14th day when on the basis of experience in other cases improvement would have been expected irrespective of splenectomy On the whole the author feels that it is preferable to treat crises by blood transfusions repeated if necessary and to postpone splenectomy until the bone marrow has recovered and the patient s clinical condition is good

Failure of Splenectomy Accounts have been published from time to time of patients reputed to suffer from hereditary spherocytosis in whom splenectomy has been a failure In retrospect it is often extremely difficult to know exactly from what the patients were suffering In most cases the diagnosis was probably hereditary non spherocytic hæmolytic anæmia or acquired hæmolytic anæmia in only a very few instances was the patient probably suffering from hereditary spherocytosis Some of the more interesting reports are referred to briefly below

(*a*) *Patients probably suffering from hereditary non spherocytic hæmolytic anæmias* It is now known that there are other congenital and hereditary hæmolytic diseases which differ fundamentally from hereditary spherocytosis in pathogenesis but which have been confused with it in the past Probably the commonest type of atypical hereditary hæmolytic anæmia for which splenectomy has been carried out is the non spherocytic type (see p 185) Dacie and colleagues (1953) described for instance four patients all of whom had had their spleens removed without benefit It is probable too that the patients who failed to respond to splenectomy described by Edwards (1951) and Lemaire Loeper and Moschoutis (1952) belonged to this group

(*b*) *Patients suffering from acquired hæmolytic anæmia* As has already been mentioned the blood picture in acquired hæmolytic anæmia of the auto antibody type may be very similar to that in hereditary spherocytosis In particular spherocytosis may be a well marked feature of the acquired disease It is difficult too in some

cases to distinguish with certainty between the two groups by study of the histology of their spleens (Dacie 1943) The patients described by Citron (1927) Kaznelson (1934) Gripwall (1938) Thompson (1939) and Dacie (1943 Case 8) respectively as not responding to splenectomy appear in retrospect to be acquired cases

Thompson s patient was a young woman suffering from jaundice of brief duration and considerable intensity Anæmia subsided after splenectomy only to reappear again after a few days The patient died 3 months later severely anæmic and with a reticulocyte count of 90 ' At necropsy many accessory spleens measuring 1–8 cm in diameter were found in the left upper quadrant of the abdomen There was no mention of any family history of hæmolytic anæmia and although the presence of accessory spleens perhaps provided an explanation for the relapse (if the patient was in fact suffering from hereditary spherocytosis) the acute onset and extreme severity of the disease the very transitory improvement following splenectomy and the lack of a family history all point to the diagnosis of acquired hæmolytic anæmia.

West Watson and Young s case (1938) was associated with an ovarian cyst its removal resulted in dramatic improvement—splenectomy had previously proved ineffective (see Chapter 14)

(c) *Patients possibly suffering from true hereditary spherocytosis* Although some writers (e g McLaughlin 1942 Young 1947) mention that the presence of accessory spleens may occasionally be a reason for splenectomy failing to result in clinical cure there are very few wholly satisfactory reports of this in the literature

McLaughlin (1942) mentioned a patient who relapsed 2 years after splenectomy and died 5 years later At necropsy large hyperplastic lymph nodes 1–3 cm in diameter were found in the abdomen McLaughlin remarked that this was by no means as frequent a cause of recurrence as overlooked accessory spleens The diagnosis of this case is far from clear

Curtis and Movitz (1946) also reported a possible example Their patient was a child aged 4 years who underwent splenectomy in 1933 for an acute hæmolytic episode There was said to be a family history of hæmolytic anæmia but no details were given The patient was well for 4½ years following operation Then anæmia and reticulocytosis returned Laparotomy was carried out and two small accessory spleens were removed. Gradual recovery ensued and the child was reported to be well – years later Again the exact diagnosis is obscure There was no mention of spherocytosis on the other hand highly phagocytic clasmatocytes were reported to be engorged with erythrocytes in supravitally stained preparations of the spleen and splenunculi —a most unusual finding in hereditary spherocytosis

Stobie (1947) reported a highly unusual case in which splenectomy resulted apparently in seeding of the peritoneum with spleen fragments with the later development of numerous splenunculi (splenosis) and recrudescence of the patient s anæmia Although this report makes no mention of osmotic fragility studies reticulocyte counts or spherocytosis and no family study was undertaken (except for the fact that it is recorded that the patient s sister and mother had gallstones) it seems not unlikely that the patient was suffering from hereditary spherocytosis

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never be withheld from a seriously anæmic patient on the ground that a harmful reaction might occur The transfusion is much more likely to be life saving than otherwise (Dameshek 1941) As already mentioned blood transfusion is the treatment of choice for patients in aplastic crises

Steroid Therapy

Steroid therapy seems to have no place in the practical management of patients with hereditary spherocytosis except perhaps in the very rare event of a combination of auto-immune hæmolytic anæmia with hereditary spherocytosis

Colman and Finch (1956) have however shown that if very large doses of steroids are given hæmolysis may be significantly reduced Three patients were given 400 mg of cortisone daily for 15 days and one patient 20 mg of ACTH intravenously over an 8 hour period for 15 days An increase in the circulating erythrocyte volume resulted and the fæcal urobilinogen output fell to about one half the control value In one patient the survival of ^{51}Cr tagged erythrocytes was demonstrated to be significantly improved The reticulocyte counts fell during treatment and radio-iron studies confirmed a diminished rate of erythropoiesis Colman and Finch concluded that cortisone probably reduced the rate of hæmolysis by interfering with the sphering activity of the spleen The limited benefit following the large doses of steroids given to these patients the rebound phenomena following their withdrawal and the dangers attendant on steroid therapy do not encourage the writer to recommend this form of treatment

THE EFFECTS OF SPLENECTOMY

It has already been mentioned that clinical cure is the almost invariable if not the invariable result of splenectomy in hereditary spherocytosis Nevertheless hæmatological signs of the disease persist and very interesting changes take place at the time of operation and during convalescence The hæmatological changes will be dealt with first and the general effects of splenectomy particularly the possibility of increased susceptibility to infections will be considered later

Hæmatological Changes

Erythrocyte Life Span after Splenectomy It is generally thought that the erythrocytes in hereditary spherocytosis have a normal or almost normal life span when the patient s spleen has been removed (Schrumpf 1951 1956 Emerson *et al* 1956) The slight increases above the normal of both reticulocyte count and serum bilirubin level which are occasionally found (p 94) suggest however that this may not always be true Unfortunately few actual measurements of erythrocyte life span

Doan (1940) referred to a patient diagnosed as suffering from congenital hæmolytic jaundice who underwent splenectomy for a hæmolytic crisis A complete remission followed which lasted for 4 years Later hæmolytic anæmia reappeared Laparotomy revealed three small accessory spleens weighing in all not more than 5 g Sections showed many highly phagocytic clasmatocytes laden with erythrocytes The patient slowly improved following the removal of the spleens and it was not until 7 months later that the reticulocyte count fell to normal In this account there is no mention of a positive family history In retrospect it seems difficult to exclude the possibility that this patient s disorder was acquired and not congenital

A possible example of relapse after splenectomy in which accessory spleens were not present was reported by Freund (1932 Case 1) Freund s patient was a boy aged 10 years who was said to have been always jaundiced Blood tests showed microcytosis and a very great increase in osmotic fragility There was no obvious family history but the erythrocyte osmotic fragility of his mother was reported to be slightly increased Splenectomy was carried out after a temporary remission hæmolysis again became active At necropsy the most conspicuous finding was the great engorgement of the liver with blood.

More recently Loeb Seaman and Moore (1952) published what appears to be a genuine instance of relapse after splenectomy in true hereditary spherocytosis In their patient a small piece of adherent spleen was known to have been left behind at the original splenectomy The patient did well for 7 years then he relapsed Radiography with the aid of Thorotrast demonstrated a radio opaque shadow 2 cm in diameter in the region of the spleen This was thought to represent hypertrophied splenic tissue derived from the fragment left behind at the original operation

Finally Edwards (1955) reported finding a spleen of about normal size and shape in the region of the porta hepatis when operating to remove a gallstone from a patient who underwent splenectomy for unequivocal hereditary spherocytosis 15 years previously Edwards (personal communication) adds that there was some evidence of hæmolysis of moderate degree at the time of the second operation

Blood Transfusion

It has in the past been stated that transfusion in hæmolytic jaundice is likely to result in severe reactions (*e g* Dawson 1931) It is not clear whether this applies to hereditary spherocytosis In the author s limited experience it has not proved to be so In some of the recorded examples of transfusion reactions it is likely that immune iso antibodies were present other patients may have been suffering from acquired hæmolytic anæmia As normal corpuscles survive well in the recipient after transfusion there seems no reason why transfusion reactions should occur with undue frequency It is possible however that serious reactions may develop in patients in severe crises if so it must be admitted that their cause is quite obscure Transfusion should however

fragile erythrocytes in the peripheral blood previously present in small numbers and responsible for the tail of the fragility curve was found to have increased by the time the operation had been completed and to increase still further during the next 24 hours (Fig 48) The increase at the time of operation was thought to be due to manipulation and compression of the spleen forcing very fragile corpuscles into the general circulation The further increase in fragility which developed within the next 24 hours was attributed to a progressive increase in the spherocytosis of these already markedly fragile cells It was pointed

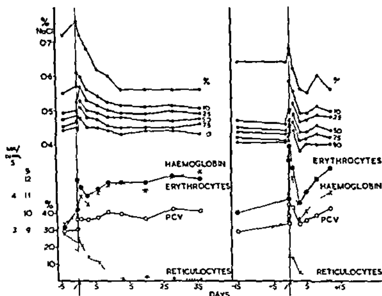


FIG 47 Changes in erythrocyte count hæmoglobin packed cell volume *reticulocyte count and osmotic fragility as the result* of splenectomy in two patients with hereditary spherocytosis (Redrawn *from Dacie (1943) Figs 3 and 4)*

out that before splenectomy such very fragile cells would almost certainly have been removed from the general circulation by the spleen Quantitative curves at 24 hours after operation showed no appreciable alteration in the point of initial lysis (the saline concentration causing 1 / lysis) By the third day after splenectomy a reduction in median corpuscular fragility (MCF) was evident and the point of initial fragility had shifted a little towards the normal indicating a disappearance of the most fragile cells During the latter half of the first week the MCF of all the patients moved back towards the pre operative level the tails of the curves having by then largely disappeared By the tenth day the shape of the curves was generally of the normal almost vertical

after splenectomy have so far been made but the data already available support the view that while this may be normal in most instances it is significantly impaired although easily compensated in others (Motulsky Giblett, *et al* 1955)

Changes in Erythrocyte Count and Hæmoglobin Detailed studies of the changes in erythrocyte counts and hæmoglobin levels during and immediately after splenectomy were made by Doan Curtis and Wiseman (1935) and Sharpe McLaughlin and Cunningham (1939)

Sharpe McLaughlin and Cunningham carried out serial blood counts at short intervals on eight patients and found that on an average the erythrocyte counts rose by about 900 000 cells per cu mm in the early stages of the operation whilst the spleen was being handled prior to its actual removal The increase in erythrocyte count continued but not so sharply for 1–2 hours after removal of the spleen and then gradually subsided the count becoming close to the pre operative level at about 48 hours after operation Thereafter a gradual rise took place the counts reaching 5 000 000 cells per cu mm in about a month One patient had a transient polycythæmia the erythrocyte count being 7 200 000 cells per cu mm 3 months after splenectomy Sharpe McLaughlin and Cunningham made the additional point that the rises in erythrocyte counts at the time of operation did not occur to anything like the same extent in atypical hæmolytic anæmia Presumably the relatively great rise in the counts in typical hereditary spherocytosis is the consequence of the extraordinary degree of congestion of the spleen in that disease Sharpe and his co workers also reported dramatic temporary increases in leucocyte counts maximal in 4–8 hours the rises were much less marked in their atypical cases The sequence of events in two of the present author s cases of hereditary spherocytosis is illustrated in Fig 47

It is generally agreed that the late results of splenectomy are excellent and that the patients erythrocyte counts and hæmoglobin levels remain within the normal range for the rest of their lives The author s own data are summarized in Table 5 (p 94)

Osmotic Fragility after Splenectomy The effect of splenectomy on erythrocyte osmotic fragility has been repeatedly studied Most authors have reported a moderate increase in resistance after operation but rarely a return to normal The early literature was reviewed by Meulengracht (1922) and some additional observations were referred to by Dacie (1943) More recently Young Izzo and Platzer (1951) Emerson (1954) and Emerson and co workers (1956) have published additional data

Dacie (1943) studied osmotic fragility changes in detail In seven patients he observed transient increases in fragility 24 hours after opera tion in two out of three patients examined the proportion of markedly

type with the median fragility at about the pre-operative level The curves remained at about this level thereafter

The regular loss of the tails of fragile cells after splenectomy suggests that before operation the tails are produced by erythrocytes which at one time trapped in the spleen and made more spherocytic have escaped again into the peripheral circulation Loss of the tails of fragility curves after splenectomy was also reported by Waugh and Lamontagne (1940) in one case and more recently by Young Izzo and Platzer (1951) and Emerson and co-workers (1954 1956)

In patients with slight to moderate increases in osmotic fragility and whose fragility curves are more nearly normal in shape little or no reduction in initial fragility may result from splenectomy Indeed the tendency seems to be for the initial fragility to be slightly increased In Fig 49 are shown the changes resulting from splenectomy in 14 patients recently studied by the author The results have been plotted from left to right in descending order of fragility the first five results demonstrate loss of tails of very fragile cells the last four show slight or moderate increases The MCF s did not change greatly in eight patients there were slight to moderate increases The mean MCF before splenectomy was 0 481 % NaCl (16 observations) and after splenectomy 0 483% NaCl (25 observations)

Young Izzo and Platzer (1951) in addition to studying the effect of splenectomy on the osmotic fragility of fresh blood also recorded mechanical fragilities and the osmotic fragility of incubated blood after splenectomy The results of both types of tests remained abnormal After splenectomy the mechanical fragility of fresh and incubated blood was slightly less than in patients before splenectomy but the increase in osmotic fragility on incubation was slightly greater in the splenectomized compared with the non splenectomized patients They also recorded transient increases in osmotic fragility of unincubated blood immediately following splenectomy in one well studied case The present author s data indicate that after splenectomy the increase in osmotic fragility resulting from incubation is usually greater than before (Figs 43 and 50)

The careful and detailed studies of Emerson and his colleagues (1954 1956) have done much to explain these earlier observations They have utilized the increment hæmolysis plot method of recording results of osmotic fragility tests (see Fig 2° p 40) These plots show that tailed curves give rise to a bimodal distribution of the osmotic fragility of the erythrocyte population and that after splenectomy with loss of the tail of the curves the increment hæmolysis plot becomes symmetrical and unimodal (Fig 39 p 96) Their studies carried out on blood from the splenic vein and the spleen pulp confirmed that such blood has a far higher proportion of highly fragile cells than has the peripheral blood and they concluded that the spleen has a predilection for cells with an osmotic fragility greater than 0 5 % NaCl and that such cells are sequestrated and made more fragile in the spleen (see also p 136) The removal of fragile cells by the spleen and the subsequent escape of a few of them having been made much more fragile was considered to be the explanation for the long tailed curves so characteristic of many pre splenectomy osmotic fragility curves After splenectomy the distribution of osmotic fragility of the erythrocyte population is restored to its basic symmetrical pattern within 2 weeks

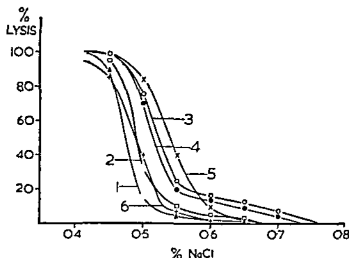


FIG 48 Changes in the osmotic fragility of the peripheral blood following splenectomy in a patient with hereditary spherocytosis (1) Before anæsthetization (2) after induction of anæsthesia (3) blood from splenic vein (4) at completion of operation (5) 24 hours after operation and (6) 5 days after operation

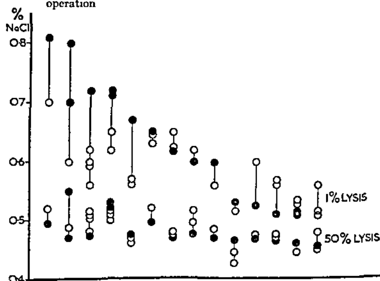


FIG 49 Changes in osmotic fragility as the result of splenectomy in 14 patients with *hereditary spherocytosis*

● = pre splenectomy ○ = post splenectomy

The concentration of sodium chloride giving 1 / lysis (initial fragility—upper series of observations) and 50 / lysis (MCF—lower series of observations) are recorded

etc) (Fig 46) This like the loss of the tails of fragile cells in osmotic fragility curves can be attributed to the absence from the circulation of erythrocytes previously trapped in the spleen and made more fragile thereby

As after splenectomy in normal subjects or in people suffering from other diseases *Howell-Jolly bodies* begin to appear in the peripheral circulation a day or so after splenectomy thereafter they may be found in small numbers for the rest of the patient s life *Target cells* observed in normal subjects and others after splenectomy however are not usually recognizable in post splenectomy films of the blood of patients with hereditary

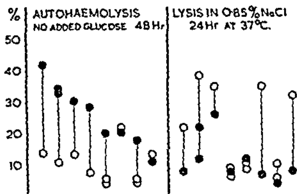


FIG 50 Effect of splenectomy on autohæmolysis (hæmolysis after 48 hours incubation at 37 C) and osmotic fragility after 24 hours incubation at 37 C (/ lysis in 0 85 NaCl) Eight patients with hereditary spherocytosis studied before (●) and after (O) splenectomy

spherocytosis—presumably this change is prevented by the persisting tendency to spherocytosis *Siderocytes* occur after splenectomy in variable numbers in the peripheral blood (2–45% mean 10% Douglas and Dacie 1953) One patient of Douglas and Dacie s series was especially interesting for large numbers of siderocytes appeared as a transient phenomenon in the peripheral blood shortly after splenectomy Within a few weeks they had practically disappeared Another feature of post splenectomy blood is an increased tendency for the erythrocytes to appear crenated in stained dried films (Fig 46) This seems to be commonly found after splenectomy irrespective of the reason for the operation

Reticulocyte Counts after Splenectomy It is generally

Emerson and his colleagues (1954 1956) confirmed the presence during the first day or two after splenectomy of substantial numbers of unusually fragile cells which they attributed as did Dacie (1943) to manipulation and compression of the spleen during its removal

Complete restoration of osmotic fragility to within the normal range as the result of splenectomy in patients whose blood previously was abnormally fragile suggests that the patient was not in fact suffering from hereditary spherocytosis Young (1955a) has reported three such patients (all with negative family histories but otherwise apparently suffering from the typical disease) whose erythrocyte osmotic fragility returned to normal within one year of splenectomy All three patients responded well clinically and their anæmia was cured It is uncertain how such cases should be classified

Autohæmolysis after Splenectomy Selwyn and Dacie (1954) reported that the mean rate of autohæmolysis at 48 hours was more than halved in four patients with hereditary spherocytosis after splenectomy Young and his colleagues (1956) however found no significant alterations The author s more recent observations appear to confirm the original data of Selwyn and Dacie The mean lysis at 48 hours (without added glucose) in 21 patients before splenectomy was 28 5% range 7 5–47 5% while that of 12 patients after splenectomy was 14 6% range 4 6–43% (Fig 43 p 102)

The correlation between autohæmolysis at 48 hours and osmotic lysis in 0 85 % NaCl at 24 hours persists after splenectomy ($r = 0\ 8142$ $P < 0\ 01$) As already mentioned the osmotic fragility (at 24 hours) is generally distinctly greater than before splenectomy (Fig 43 p 102) The changes in a small series of eight patients studied both before and after splenectomy are shown in Fig 50 Whereas autohæmolysis at 48 hours is significantly reduced in six patients osmotic fragility (% lysis in 0 85% NaCl) is increased in six patients These trends are responsible for the almost complete separation of the two series of data shown in Fig 43

It is not known for certain why the relationship between autohæmolysis and the osmotic lysis in 0 85% NaCl of incubated erythrocytes is altered as the result of splenectomy Before splenectomy two populations of cells are circulating normal spherocytes and markedly spherocytic cells which have escaped from the spleen Possibly it is the latter type of cell which undergoes such marked autohæmolysis before splenectomy The increased osmotic fragility on incubation following splenectomy is perhaps due to the circulation of cells which formerly would have been taken out of circulation by the spleen

Morphology of the Erythrocytes after Splenectomy It is generally agreed that the microcytosis and spherocytosis persist after splenectomy but that there is some return towards the normal as the result of the loss of the most markedly spherocytic cells (Hawksley 1936 Vaughan 1937 Meulengracht 1938

Leucocyte Counts after Splenectomy

Absolute or relative lymphocytosis has been reported by Gännslen (1936) and Olmer Mongin and Carcassonne (1954) Gännslen commented that this was remarkable in view of the large amount of lymphatic tissue removed at operation This reaction is however not confined to hereditary spherocytosis A tendency to neutropenia with relative or absolute lymphocytosis and monocytosis seem to be the usual results of splenectomy carried out for a variety of causes or even for traumatic rupture (Singer Miller and Dameshek 1941 Ek and Rayner 1950)

General Effects of Splenectomy

Splenectomy is invariably followed by improvement in general well being Adult patients usually feel entirely normal and appear to be so except for perhaps traces of jaundice in a small minority of patients Children who occasionally appear to be retarded either mentally or physically can be expected to develop normally subsequently (Bernard Boiron and Estager 1952)

Possible Decreased Resistance to Infection Although there were a few reports in the literature of tuberculosis developing after splenectomy for hereditary spherocytosis (*e g* Beckman and Jaderholm 1931–39) the possibility that the infection had any thing to do with the removal of the spleen *per se* has been generally discounted However the whole subject of a possible increased proneness to infection after splenectomy particularly in infants was recently reopened by the publication of King and Shumacker (1952) In reviewing the results of 100 splenectomies they found that five of the patients had developed severe infections subsequently All five were infants less than 6 months of age probably suffering from hereditary spherocytosis four of them contracted meningococcal meningitis or meningococcæmia the fifth died of an illness thought to be septicæmia Several other papers have since been published on the incidence of infections following splenectomy and as the subject is an important one they are considered briefly below

Walter and Chaffin (1955) reviewed the results of 7- splenectomies Hereditary spherocytosis was the diagnosis in 37 patients of which ten were under 7 months of age No serious infections were encountered in a follow up extending from 10 months to 13 years

Gofstein and Gellis (1956) on the other hand concluded that there probably was a positive correlation between serious infections and splenectomy although the incidence of infection did not seem to be as high as the figures of King and Shumacker (1952) had suggested The results of 62 splenectomies carried out for congenital hæmolytic anæmias were reviewed ten of the patients were infants less than 6 months of age and seventeen were under 1 year none suffered from serious infections

believed that reticulocyte counts fall to normal after splenectomy in patients with hereditary spherocytosis Young Izzo and Platzer (1951) reported an average of 0 85% reticulocytes with a range from 0 2 to 2 1% in twelve patients all examined a year or more after splenectomy There are however a few records of reticulocyte counts remaining slightly raised Dacie (1943) found that *in six of twelve patients examined between 3 months and 6 years after splenectomy* the counts ranged from 1 5 to 3 6% The counts in 32 patients are recorded in Table 5 (p 94) the mean count was 1 3% and in four patients the count exceeded 2 5% Counts exceeding 2 5% have also been reported by Gripwall (1938) Thompson (1939) and Singer Miller and Dameshek (1941) It is fair to say however that great care in counting is necessary after splenectomy for it is difficult to differentiate with certainty cells containing very small amounts of reticulo filamentous material from siderocytes containing Pappenheimer bodies which also stain with brilliant cresyl blue

Bile Pigment Metabolism after Splenectomy The plasma bilirubin concentration falls significantly within a few days of splenectomy Whether or not the level falls to within the normal range in every case is not quite clear Meulengracht (1938) mentioned early reports of mild recurrences of jaundice after operation and Singer Miller and Dameshek (1941) gave values between 0 9 and 1 1 mg in four patients More recently Edwards (1951) reported levels between 0 8 and 1 7 mg per 100 ml in twelve patients On the other hand Young Izzo and Platzer (1951) found strictly normal values (0 1 to 0 8 mg per 100 ml) in 12 patients one or more years after splenectomy The author s own observations on 18 patients after splenectomy ranged between 0 3 and 1 8 mg with a mean (0 68 mg) towards the upper limit of the normal range (Table 5)

There are few relevant observations on the fæcal excretion of uro bilinogen after splenectomy Goldschmidt Pepper and Pearce (1915) found that the output of pigment fell after splenectomy to about one tenth of its previous level becoming very close to the normal Eppinger (1920) reported a decrease in excretion after splenectomy to one quarter of the pre splenectomy level but the excretion still remained above the normal More recently Watson (1937) reported values within the normal range in four patients after splenectomy In one patient a figure of 616 mg per day was observed 15 days after operation however the output was normal (85 mg) 2 months later Barker (1938) reported normal figures in three patients after splenectomy and Singer Miller and Dameshek (1941) subnormal values for the hæmolytic index in three patients after splenectomy and a normal value in one patient.

sufficient in itself to bring about hæmolysis by the normal hæmolytic processes of the body Troisier (1910) complicated the issue by attributing the erythrocyte fragility to the fixation on the corpuscles of hæmolysins Banti (1913a) elaborated this concept still further and postulated that the spleen was an important source of hæmolysin formation

The view that hæmolysis depended upon a primary abnormality of the erythrocytes has had many adherents (*e g* Naegeli 1931 Haden 1934 Thompson 1936 Vaughan 1936 Gripwall 1938 etc) Meulengracht (1938) on the other hand after careful weighing of the evidence favoured a hyperactive condition of the spleen as the primary and fundamental factor Dameshek and Schwartz s (1938) experiments with hæmolytic immune sera resuscitated Troisier s idea of erythrocyte damage due to hæmolysins As will be discussed later Dameshek and Schwartz were right in regard to acquired hæmolytic anæmia but wrong about the hereditary type

In the last 15 years fresh evidence has been accumulated which is strongly in favour of the hypothesis of an intrinsic corpuscular defect This evidence has been both direct and indirect transfusion experiments provided direct evidence for the intrinsic nature of the corpuscular defect by showing that the corpuscles of the patient underwent rapid hæmolysis in a normal recipient and indirect evidence by demonstrating that normal corpuscles survived normally in patients suffering from hereditary spherocytosis Dacie and Mollison s (1943) experiments moreover showed conclusively (*a*) that normal erythrocytes *were not* destroyed by the enlarged spleen of hereditary spherocytosis and (*b*) that spherocytes *were* destroyed by normal spleens This work since confirmed by many other workers indicated that the enlargement of the spleen was secondary rather than primary and that it was acting as a destroyer of abnormal corpuscles

The Nature of the Corpuscular Defect The fact that spherocytosis is a progressive process has already been remarked upon As the corpuscle circulates it probably becomes more spheroidal with a progressively decreasing surface area It now seems certain that this process is greatly accelerated when blood is stagnant within the spleen The high hæmoglobin concentration of the spherocyte its slightly diminished potassium content and its possibly low surface lipoid content have also been mentioned (p 104)

The demonstration of increased corpuscular osmotic fragility *in vitro* was never a satisfactory explanation for lysis *in vivo* for

in a 3 year follow up period One older patient of the remaining forty five died however of pneumococcal meningitis and one infant (not having congenital hæmolytic anæmia) out of a total of 17 infants splenectomized in the first year of life died of tracheobronchitis Single additional examples of fatal post operative infections in infants with hereditary spherocytosis were reported by Newns (1951) White (1956) and Robinson (1957)

Recent reviews include those of Smith Erlandson Schulman and Stern (1957) Huntley (1958) and Burman (1958) Smith and his colleagues referred to serious infections as occurring in 19 splenectomized children and adolescents 13 months to 17 years of age The majority of infections were instances of meningitis chiefly pneumococcal acute benign pericarditis (in Cooley s anæmia patients) and septicæmia Young children did not seem to be particularly susceptible but the authors contrast the occurrence of post operative infections occurring in fourteen of 50 splenectomized children at a time when 225 splenectomies were carried out in adults with only one subsequent serious infection

Huntley (1958) reported that of 46 infants and children followed up after splenectomy seven developed serious infections five were infants under 1 year of age However none of the 12 children with hereditary spherocytosis were among them and Huntley makes the point that splenectomy is hazardous chiefly in patients whose basic disease renders them susceptible to infection

The accounts reviewed above certainly do not give a clear picture of the incidence of infections after splenectomy nor of the age at which they are most likely There are no controls and the follow up periods vary from series to series It would seem wise however not to carry out splenectomy in infants with hereditary spherocytosis unless there are strong indications for the operation but it has to be admitted that the report of King and Shumacker (1952) is the only one pointing strongly to infants in the first year of life as being particularly susceptible to infection

PATHOGENESIS OF HEREDITARY SPHEROCYTOSIS

From the very first most writers have linked the presence of excessive hæmolysis with an abnormality of the patient s erythro cytes Vanlair and Masius (1871) for instance quite correctly suggested that the microcytes they observed were senile erythrocytes on the way to destruction (globules atrophiques) and compared the microcytosis with that caused by heating blood Chauffard s (1907) discovery of the increase in osmotic fragility (and his rediscovery of the microcytosis) led to the concept of fragilité globulaire as the cause of the hæmolysis *in vivo* Widal and his associates (Widal and Philibert 1907) believed that the abnormal corpuscular fragility was the primary factor and

Giblett *et al* 1955 Tabechian Altman and Young *1956* Prankerd 1957 1959)

Prankerd Altman and Young (1954 1955) demonstrated when blood from patients with hereditary spherocytosis was incubated with radio active phosphorus (^{32}P) that although the uptake of ^{32}P was normal more inorganic phosphate was formed relative to 2 3-diglycerophosphate (2 3 DPG) and adenosine triphosphate (ATP) These abnormalities persisted after splenectomy and in most cases but not all were found to be largely abolished and the partition of ^{32}P restored to normal by incubation with adenosine Young (1955a) in a later report stated that the addition of adenosine restored the phosphorous metabolism to normal in eighteen out of 20 patients The data of Motulsky Giblett and their colleagues (1955) are in line with those of Prankerd and Young and co workers They too found that the total ^{32}P uptake and glycolytic rate of the hereditary spherocyte were normal and that the metabolic lesion of the cells was reversible by excess glucose and adenosine

Bertles (1957) found by measurements *in vitro* that the rate of sodium transport across the surface membranes of hereditary spherocytes was generally considerably increased The transport rates of three patients each of whom had normal parents were the lowest of the ten patients studied and this Bertles considered was consistent with the hypothesis that the term hereditary spherocytosis covers more than one genetically determined biochemical abnormality

The exact abnormality or abnormalities in the hereditary spherocyte have not yet been defined Tabechian Altman and Young (1956) found that the cells were unusually sensitive to the action of fluoride in inhibiting orthophosphate exchange and *suggested that the magnesium dependent enzymes enolase and* ATPase are defective Prankerd (1957 1959) concluded that the metabolic block might involve phosphofructokinase or enolase

The significance of these facts is not yet clear Somewhat similar but not identical abnormalities have been demonstrated with the erythrocytes of patients with auto immune hæmolytic anæmia and myeloid metaplasia (myelosclerosis) Only in hereditary spherocytosis however does adenosine exert a favourable effect (Young 1955b) Presumably in hereditary spherocytosis the essential genetically controlled lesion is basically a relative slowness in the rate of formation of ATP and/or other organic phosphorus compounds Prankerd Altman and Young (1955) even suggested that an adequate rate of regeneration of ATP *thus ensuring an adequate availability of energy rich* phosphate bonds was necessary for the maintenance of the biconcave shape of the erythrocyte It has already been mentioned that there is evidence that the lesion may not be the same in all cases of clinical hereditary spherocytosis

there never seemed any likelihood that the tonicity of the plasma would be diminished in any organ of the body sufficiently to cause osmotic hæmolysis The observations of Ham and Castle (1940a b) and of Dacie (1941) on the rapid spontaneous hæmolysis of spherocytes were particularly significant for they provided the first satisfactory demonstration of an abnormal tendency to lysis *in vitro* which might be applicable to conditions *in vivo* The subsequent demonstration of the increased sensitivity of spherocytes to mechanical trauma was less significant for in the absence of the spleen spherocytes clearly withstand the wear and tear of circulation very well indeed as the clinical results of splenectomy demonstrate

Selwyn and Dacie (1954) and Selwyn (1955) showed that the cation changes which occur when spherocytes are incubated *in vitro* in serum are not grossly abnormal and do not seem to be correlated with or responsible for the rapid lysis They considered that this was more likely to be the consequence of a membrane defect which led to a relatively rapid irreversible contraction of the surface of the cell The rapidly progressive increase in osmotic fragility on incubation was attributed to the unusual degree of cell membrane contraction The nature of the corpuscular defect was not elucidated but it was demonstrated in contrast to other types of congenital hæmolytic anæmia that glucose utilization was normal *in vitro* and that the addition of excess glucose reduced the rate of autohæmolysis substantially although not to within the normal range

Young and his colleagues (Young 1955a b Young *et al* 1956 1958) extended these observations Studying autohæmolysis they found that the addition of either glucose or mannose to defibrinated blood markedly reduced the hæmolysis of hereditary spherocytes while pyruvate had no effect Sodium fluoride and iodoacetate inhibitors of glycolysis nullified the effect of glucose Adenosine guanosine and inosine substantially reduce hæmolysis and according to Young and his colleagues (1958) they are more effective than glucose It was concluded that adenosine and guanosine reduced hæmolysis by providing substrates which could be metabolized for continuing glycolysis

More recently the use of isotopic and chromatographic techniques has demonstrated previously unsuspected biochemical abnormalities in the hereditary spherocyte which seem likely to be the basis of the reduced life span *in vivo* and rapid autohæmolysis *in vitro* (Prankerd Altman and Young 1954 1955 Prankerd 1955 Motulsky Gabrio Burkhart and Finch 1955 Motulsky

fragility of the erythrocytes is increased in some way by their passage through the spleen There is evidence which suggests that the circulation of blood through the spleen in hereditary spherocytosis is slow (see below) It seems likely that the slowness of the circulation is of major pathogenetic importance for the changes which occur in blood incubated at 37° C *in vitro* and which eventually lead to hæmolysis may well take place *in vivo* in blood stagnant in the spleen

The evidence for the stagnation of blood within the spleen in hereditary spherocytosis is admittedly circumstantial However inspection of a section of a spleen with its pulp cords typically grossly engorged with blood (Fig 45) leads to the conclusion that there is so much blood present that it would be impossible for it all to escape quickly from the spleen pulp into the relatively small venous channels Support for this view was obtained by the author when he found that it took far longer to wash the spleens from patients with hereditary spherocytosis free from blood by saline perfusion through the splenic artery than in comparable experiments carried out with control spleens This seemed likely to be due to the fact that the spleen in hereditary spherocytosis contained so much more blood initially Dacie (1943) however found that large oval fowl cells were able to pass through the spleen (in small numbers) as rapidly as through normal spleens and concluded that there was no anatomical block to the circulation through the spleen in hereditary spherocytosis It seemed likely nevertheless that much of the pulp was a backwater outside the main current of the blood stream

// Further direct confirmation of the hæmolytic action of the spleen in hereditary spherocytosis has more recently been provided by the observation that radioactivity can be detected in relatively large amounts by surface counting over the spleen within a few days of labelling the patient s erythrocytes with radioactive chromium (Jandl *et al* 1956 Hughes Jones and Szur 1957 Schloesser *et al* 1957 Fig 32 p 66)

The above mentioned observations to say nothing of the indirect evidence of the good clinical results of splenectomy unquestionably point to the spleen as a most important factor in the pathogenesis of hereditary spherocytosis It seems too highly probable that the congestion and stagnation of blood within the spleen are closely linked to the mechanism of splenic hæmolysis If this is conceded there nevertheless still remains the problem as to exactly how the congestion is brought about and how stagnation within the spleen leads to the destruction of the erythrocytes It has to be borne in mind that splenic congestion is also very frequently found in other types of hæmolytic anæmias as well as in hereditary spherocytosis Thus congestion with blood may be the most

The Hæmolytic Action of the Spleen Direct evidence for the hæmolytic activity of the spleen is provided by observations on the bilirubin content of splenic vein blood Values considerably higher than in the peripheral blood have been found (see Gripwall 1938 and Fig 51) Similarly the osmotic fragility of blood from

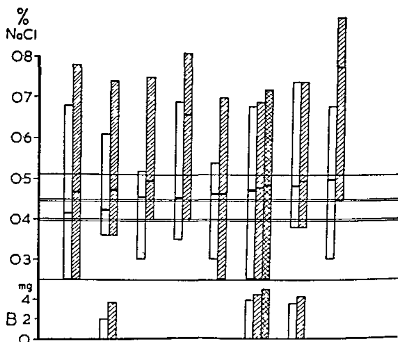


FIG 51 Osmotic fragility measurements and serum bilirubin concentrations (B) on peripheral and splenic vein blood samples from eight patients with hereditary spherocytosis
The splenic vein samples were taken from the excised spleen as soon as possible after excision
Open rectangles represent peripheral blood samples hatched rectangles represent splenic vein samples the cross hatched sample was obtained from the spleen pulp (See also Fig 39)

the splenic veins or more particularly from the spleen pulp has been found to be greater than that of blood taken from the peripheral circulation (MacAdam and Shiskin 1922–23 Campbell and Warner 1925–26 Gripwall 1938 Young Platzer Ervin and Izzo 1951 Weisman Hurley Harris and Ham 1953 Emerson 1954 Weisman Ham Hinz and Harris 1955 Emerson *et al* 1956) (Fig 51) There can be no doubt therefore that the osmotic

lysis in a sodium chloride solution exceeding 0·5% in concentration were particularly sensitive to trapping in the spleen

The last point to be considered is the way in which the spleen brings about erythrocyte destruction As compared with spleens removed from patients with acquired hæmolytic anæmia erythrophagocytosis is not conspicuous It seems likely to the author and to Young Platzer Ervin and Izzo (1951) and Emerson and co workers (1950) that lysis of the stagnant blood takes place by the mechanisms which produce lysis of incubated blood *in vitro* As already mentioned this cannot be satisfactorily explained by the accumulation of osmotically active metabolites which cause swelling and eventually osmotic lysis Nor does it seem likely that the lysis can be explained by an accumulation in the stagnant blood of potentially hæmolytic substances such as lysolecithin (Cripwall 1938 Fåhraeus 1939) or other tissue lysins (see (Ponder 1951) to which hereditary spherocytes are possibly peculiarly sensitive Attempts to demonstrate an increased formation of lysolecithin in spleen blood have been unsuccessful (Singer 1941) It seems most likely that the lysis is the consequence of the defective intracellular metabolism of the spherocyte (see p 133) which although unimportant in relation to the cell s survival in the circulating blood brings about irreversible damage and eventual lysis if and when the cell is trapped in the spleen

How trapping of spherocytes in the spleen pulp brings about lysis is not clearly known Stagnation of blood in the spleen pulp and the hæmoconcentration which results from the filtering action of the spleen probably reduce significantly the amounts of metabolizable substrate including glucose available to individual erythrocytes It may well be that under these conditions the hereditary spherocyte because of its genetically determined metabolic handicaps (Young 1955b) cannot maintain sufficient energy production to maintain its discoidal shape and the integrity of its surface Increased spherocytosis and ultimate lysis are the result The phenomenon of rapid autohæmolysis *in vitro* is probably brought about in a similar way

To sum up recent work suggests that the excessive rate of hæmolysis in hereditary spherocytosis is due fundamentally to the production of erythrocytes with a genetically determined defect in intracellular metabolism The exact nature and extent of the metabolic defect(s) have not yet been fully demonstrated Morphologically the erythrocytes in hereditary spherocytosis are best described as microspherocytes cells smaller in diameter but thicker than normal erythrocytes These microspherocytes

striking pathological change in acquired hæmolytic anæmia (Dameshek and Schwartz 1940) (see also Chapter 11) it is also characteristically found after the administration of hæmolytic sera or poisons to laboratory animals (Banti 1913a Eppinger 1920) It seems likely that the congestion is due to the spleen s remarkable property of filtering off from the blood stream damaged or abnormal corpuscles irrespective of whether the damage is due to the effects of immune antibodies hæmolytic poisons or whether the erythrocytes are inherently abnormal as in hereditary spherocytosis

Recently it has been shown experimentally by differential agglutination that the spherocytes of hereditary spherocytosis are more easily trapped in the spleen than are normal corpuscles (Emerson Shen and Castle 1946 Emerson *et al* 1947 Young 1947 Young *et al* 1951 Weisman *et al* 1953 1955) Young and his colleagues (1951) perfused *in vitro* with a mixture of normal erythrocytes and hereditary spherocytes freshly removed spleens from three patients with thrombocytopenic purpura and found that the spherocytes were selectively removed from the perfusate Weisman and his colleagues (1955) carried out a similar experiment *in vivo* They transfused a patient suffering from thrombocytopenic purpura with hereditary spherocytosis blood shortly before the recipient underwent splenectomy 49% of the blood obtained from the spleen after operation was identified as hereditary spherocytosis (donor) blood compared with 3% in the general circulation The donor blood from the spleen had had its osmotic fragility markedly increased and Weisman and his colleagues pointed out that this was further evidence that the normal spleen is able to condition spherocytes after filtering them off from the circulation

✓ The problem as to how the congestion in the spleen is brought about has not been solved Various hypotheses have however been put forward Klemperer (1938) suggested that the presence of abnormal corpuscles reflexly initiated arterial vasodilatation and Whipple (1941) postulated that the stagnation was due to ✓the abnormal shape of the corpuscles *i e* the spherocytosis Whipple considered that whereas normal discoidal cells probably could circulate through the spleen without difficulty spherocytes because of their shape might find it difficult to traverse the slit like stomata leading from the pulp into the splenic sinuses This hypothesis is attractive but difficult to confirm or refute It assumes that the erythrocytes lie mostly in the pulp during life Emerson and co workers (1956) concluded that cells undergoing

lysis in a sodium chloride solution exceeding 0 5% in concentration were particularly sensitive to trapping in the spleen

The last point to be considered is the way in which the spleen brings about erythrocyte destruction As compared with spleens removed from patients with acquired hæmolytic anæmia erythrophagocytosis is not conspicuous It seems likely to the author and to Young Platzer Ervin and Izzo (1951) and Emerson and co-workers (1956) that lysis of the stagnant blood takes place by the mechanisms which produce lysis of incubated blood *in vitro* As already mentioned this cannot be satisfactorily explained by the accumulation of osmotically active metabolites which cause swelling and eventually osmotic lysis Nor does it seem likely that the lysis can be explained by an accumulation in the stagnant blood of potentially hæmolytic substances such as lysolecithin (Gripwall 1938 Fåhraeus 1939) or other tissue lysins (see (Ponder 1951) to which hereditary spherocytes are possibly peculiarly sensitive Attempts to demonstrate an increased formation of lysolecithin in spleen blood have been unsuccessful (Singer 1941) It seems most likely that the lysis is the consequence of the defective intracellular metabolism of the spherocyte (see p 133) which although unimportant in relation to the cell s survival in the circulating blood brings about irreversible damage and eventual lysis if and when the cell is trapped in the spleen

How trapping of spherocytes in the spleen pulp brings about lysis is not clearly known Stagnation of blood in the spleen pulp and the hæmoconcentration which results from the filtering action of the spleen probably reduce significantly the amounts of metabolizable substrate including glucose available to individual erythrocytes It may well be that under these conditions the hereditary spherocyte because of its genetically-determined metabolic handicaps (Young 1955b) cannot maintain sufficient energy production to maintain its discoidal shape and the integrity of its surface Increased spherocytosis and ultimate lysis are the result The phenomenon of rapid autohæmolysis *in vitro* is probably brought about in a similar way

To sum up recent work suggests that the excessive rate of hæmolysis in hereditary spherocytosis is due fundamentally to the production of erythrocytes with a genetically-determined defect in intracellular metabolism The exact nature and extent of the metabolic defect(s) have not yet been fully demonstrated Morphologically the erythrocytes in hereditary spherocytosis are best described as microspherocytes cells smaller in diameter but thicker than normal erythrocytes These microspherocytes

striking pathological change in acquired hæmolytic anæmia (Dameshek and Schwartz 1940) (see also Chapter 11) it is also characteristically found after the administration of hæmolytic sera or poisons to laboratory animals (Banti 1913a Eppinger 1920) It seems likely that the congestion is due to the spleen s remarkable property of filtering off from the blood stream damaged or abnormal corpuscles *irrespective of whether the damage is due* to the effects of immune antibodies hæmolytic poisons or whether the erythrocytes are inherently abnormal as in hereditary spherocytosis

Recently it has been shown experimentally by differential agglutination that the spherocytes of hereditary spherocytosis are more easily trapped in the spleen than are normal corpuscles (Emerson Shen and Castle 1946 Emerson *et al* 1947 Young 1947 Young *et al* 1951 Weisman *et al* 1953 1955) Young and his colleagues (1951) perfused *in vitro* with a mixture of normal erythrocytes and hereditary spherocytes freshly removed spleens from three patients with thrombocytopenic purpura and found that the spherocytes were selectively removed from the perfusate Weisman and his colleagues (1955) carried out a similar experiment *in vivo* They transfused a patient suffering from thrombocytopenic purpura with hereditary spherocytosis blood shortly before the recipient underwent splenectomy 49% of the blood obtained from the spleen after operation was identified as hereditary spherocytosis (donor) blood compared with 3% in the general circulation The donor blood from the spleen had had its osmotic fragility markedly increased and Weisman and his colleagues pointed out that this was further evidence that the normal spleen is able to condition spherocytes after filtering them off from the circulation

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perhaps because of their abnormal shape are selectively filtered off from the circulation by the spleen There they undergo further sphering and ultimate lysis probably because their metabolic defects become of critical importance under conditions of circulatory stagnation Splenectomy cures the disease clinically and the rate of in vivo hæmolysis becomes normal or almost normal The fundamental biochemical and morphological abnormalities of the patient's erythrocytes are nevertheless not significantly affected they are however of no clinical importance once the spleen has been removed

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CHAPTER 3

THE CONGENITAL HÆMOLYTIC ANÆMIAS
2 HEREDITARY ELLIPTOCYTOSIS

The presence of elliptical erythrocytes in man was first described by Dresbach in 1904 in the blood of a mulatto Since then the condition has been observed in many races throughout the world and it now has an extensive literature (see Wyandt Bancroft and Winship 1941 Penfold and Lipscomb 1943 Bernard Dugas and Cotlenko 1953 Wolman and Özge 1957 Baurle and Reissner 1958) Bishop (1914) observed elliptocytes in the blood of two siblings belonging to the same family and it is now known that the abnormality (hereditary elliptocytosis[1]) is genetically determined The incidence of hereditary elliptocytosis is not known for certain it is uncommon but not rare Wyandt Bancroft and Winship (1941) excluding the large family they were studying found two additional cases in 7 000 examinations and concluded that the frequency in the general population in the United States was about 4 per 10 000

Hereditary elliptocytosis like hereditary spherocytosis is associated with increased hæmolysis but, unlike hereditary spherocytosis signs of overt hæmolysis are found in a small minority of cases only It is in fact only recently that cases associated with hæmolytic anæmia have been at all widely recognized and even in the 1940 s some writers e g Wyandt Bancroft and Winship (1941) and Hedenstedt (1947) concluded that there was no direct relationship between elliptocytosis and anæmia It is now however generally agreed that it is possible to divide cases of hereditary elliptocytosis into three categories those with no signs of hæmolysis those with compensated hæmolysis and those with non compensated hæmolysis

The relative frequency of the three catagories is not yet known The incidence of major or minor degrees of hæmolysis seems to vary from family to family Penfold and Lipscomb (1943) in

[1] Ovalocytosis " at one time frequently used seems now to have been generally superseded by elliptocytosis which more correctly describes the shape of the corpuscles. Hereditary Ovalocytosis was however the term recommended by the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood forming Organs (1950)

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first time become manifest as the result of an intercurrent infection Rarely the disorder leads to severe anæmia in infancy (e g Case 11 of Dacie Mollison Richardson Selwyn and Shapiro 1953 Lipton 1955) Gallstones are a not infrequent complication and chronic leg ulcers as in hereditary spherocytosis have been reported (Motulsky Singer Crosby and Smith 1954 Duela Soares and Parreira 1958) Tower skull and other skeletal abnormalities have occasionally been observed (Bernard Dugas and Cotlenko 1953)

The spleen is usually palpable in patients who present with clear signs of increased hæmolysis in elliptocytosis trait it is not palpable and probably not enlarged

Blood Picture in Hereditary Elliptocytosis

Morphology of Elliptocytes[1] The degree of elliptocytosis varies not only from subject to subject but also within the cell population of any particular person (Figs 55–61 63–66) Usually up to 90% of the cells are affected some being markedly elongated others merely oval

Poikilocytes microcytes and cell fragments are inconspicuous except in patients in whom there is evidence of increased hæmolysis There is however no clear relationship between the degree of elliptocytosis and the presence or absence of hæmolysis Microspherocytes are commonly found in small numbers in patients with active hæmolysis although they are not necessarily present They are usually oval in contour not round as in hereditary spherocytosis (e g Figs 59 and 64)

The nucleated precursors of elliptocytes are round and reticulocytes are also round or at any rate conspicuously less elliptical than adult corpuscles (Florman and Wintrobe 1938) The elliptocytosis is not fully evident at birth but increases to a maximum by about the end of the third month (Hunter 1932–33 Helz and Menten 1944)

Elliptocytes have been classified on the basis of diameter measurements (e g by Gunther 1928 Lambrecht 1938 Guasch and Raichs 1949 Zini and Leubner 1951) and the distinction between hereditary elliptocytosis and normal blood which may contain up to 10–15% of definitely elliptic cells has been carefully defined (Florman and Wintrobe 1938 Hedenstedt 1947) (see *Diagnosis of Hereditary Elliptocytosis* p 165) In elliptocytosis trait the erythrocyte counts are usually within the normal range

[1] Although superficially resembling the oval erythrocytes of the *Camelidæ* the two types of cell differ markedly in their reactions *in vitro* (Jung 19 7)

reviewing 400 cases of elliptocytosis reported in the literature concluded that in 12% of them there had been signs of increased hæmolysis The probability is that the percentage would have been higher if erythrocyte life span measurements had been carried out

Inheritance of Hereditary Elliptocytosis Inheritance follows the Mendelian dominant pattern (Wyandt Bancroft and Winship 1941) and the gene or genes for elliptocytosis appear to be located in some families at least on the same chromosome as that carrying the gene(s) for the Rh blood group system (Goodall Hendry Lawler and Stephen 1953 Marshall Bird Bailey and Beckner 1954 Morton 1956) The expression of the gene for elliptocytosis appears to vary widely as is shown by variation from case to case in the degree to which the erythrocytes are elliptocytic and also by the extent to which the morphological abnormality is associated with increased hæmolysis As already mentioned the presence of hæmolysis often appears to be a family characteristic (*e g* Cases 1–3 p 161) Whether an additional abnormal gene is involved in such cases is not yet known It seems equally probable that hereditary elliptocytosis in its various clinical forms is determined by a series of abnormal allelic genes (*cf* the abnormal hæmoglobin syndromes)

The vast majority of subjects with hereditary elliptocytosis appear to be heterozygous for the gene for elliptocytosis even if they present with signs of excessive hæmolysis Nevertheless a few probable homozygotes have been reported This interesting group of patients who had severe hæmolytic anæmia are referred to in more detail on p 161

CLINICAL AND HÆMATOLOGICAL FEATURES OF HEREDITARY ELLIPTOCYTOSIS

Symptoms and Signs As already mentioned hereditary elliptocytosis exists usually as a harmless and symptomless trait (elliptocytosis trait) discovered perhaps as the result of a routine blood test On the other hand the presence of increased hæmolysis leads to a variety of signs and symptoms according to its severity The clinical syndromes are in fact indistinguishable from those of hereditary spherocytosis Anæmia may be severe or in compensated cases absent Most patients will however be found to be mildly to moderately anæmic and to be slightly jaundiced from time to time In some *e g* the patient described in the first edition of this book (p 99) overt hæmolysis may for the

Table 6

Hæmatological Data in Hereditary Elliptocytosis

Family	Erythrocytes (minimum counts) (mill /cu mm)	Hæmoglobin (minimum values) (g /100 ml)	MCV (mean) (cu μ)	MCHC (mean) (/)	Reticulocytes (maximum counts) ( )	Serum bilirubin (maximum values) (mg /100 ml)	Erythrocyte life span (mean) (days)
A Mrs La	3 4	11 0	100	31 5	17 0	1 9	
Mr Lo (trait)	4 6	15 4	98	34	1 6		
T La (trait)	4 5	14 7	92	35	2 7		
J La (trait)	4 6	13 0	84	34	1 8		
B D Hi *	3 4	11 4	61	34	3 7	0 3	
Mrs Hi (trait)	4 3	13 6	94 5	32	2 3	0 4	
M Hi (trait)	4 0	12 7	87 5	34	4 4	0 4	
C B A (Case 1)	2 7	9 1	101 5	33	17 0	1 6	14
J A (Case 2)	3 2	10 6	100	33	12 0	3 2	
Mrs A (Case 3)	3 7	11 5	100	31	2 9	0 4	
D I Hw		12 0		32	9 0	2 0	21
Mrs M (trait)		14 0		32 5		0 4	
E Mrs Hl *		14 5		33	4 5		
F G K		13 5		33	4 1	0 6	
Mrs K (trait)		13 5		33	0 4		
G Mrs B	3 6	9 4	90	34	15 0	1 9	20
H R Kr	3 6	10 2	78	30 5	8 1		
Mrs Kr (trait)		13 6		30	2 8		

* After splenectomy

Counts above the normal have however occasionally been reported Stephens and Tatelbaum (1934–35) stated for instance that the counts of the affected members of a family averaged 6 470 000 per cu mm despite the fact that their hæmoglobin levels were normal

The mean cell volume (MCV) is usually normal or slightly above normal and the mean cell hæmoglobin (MCH) and hæmoglobin concentration (MCHC) are also usually normal (see Table 6)

The reticulocyte count may be expected to be normal in elliptocytosis trait or slightly raised in non anæmic patients with fully compensated hæmolysis In patients with overt hæmolytic anæmia the counts may reach 20% or even higher

Hæmoglobin in Hereditary Elliptocytosis All workers agree that hereditary elliptocytosis is not associated with the presence of an abnormal hæmoglobin Fœtal hæmoglobin too is usually absent except in the neonatal period White Beaven and Ellis (1959) for instance found a definite increase in Hb F (1 5%) in only one of twelve patients studied Ducla Soares and Parreira (1958) have however reported larger amounts (3 1–6 0%) in three members of a family who were exceptionally severely anæmic (for hereditary elliptocytosis) As referred to on p 167, there are well authenticated reports of the co existence of hereditary elliptocytosis and Hb S or Hb C in the same individual The effects do not seem to be summated

Osmotic Fragility Studies on osmotic fragility have given variable results In elliptocytosis trait the results seem invariably to be normal in the presence of hæmolysis osmotic fragility may or may not be increased The author s own observations are illustrated in Fig 52 The effect of incubation at 37 C for 24 hours has seldom been studied The author s data suggest that in patients whose osmotic fragility is increased before incubation (and in whose films oval microspherocytes can be seen) the effect of incubation will be similar to that in hereditary spherocytosis *i e* hæmolysis will take place in 0 85% NaCl It is clear however that unlike in hereditary spherocytosis the osmotic fragility after incubation may not be increased above the normal despite the presence of overt hæmolysis *in vivo* (Fig 52 Family A (Mrs La))

Mechanical Fragility Few observations have been made but where spherocytes or schistocytes are present increased mechanical fragility can be expected (*e g* Case 11 of Dacie *et al* 1953)

considerably as in hereditary spherocytosis These data are shown in Table 7

Table 7
Autohæmolysis in Hereditary Elliptocytosis

Family	% Hæmolysis			
	24 hr (No added glucose)	24 hr (With added glucose)	48 hr (No added glucose)	48 hr (With added glucose)
A Mrs La	0 3	0 1	2 9	1 0
Mr Lo (trait)	0 4	0 1	3 5	0 8
B D Hi *	1 5	1 4	3 0	17 0
Mrs Hi (trait)	<0 1		1 7	
C B A † (Case 1)	1 4	0 3	2 0	1 7
J.A (Case 2)	2 7	1 0	10 5	6 3
Mrs A † (Case 3)	0 4	0 2	4 5	0 2
D P Hw	1 1	0 2	0 9	0 8
Mrs M (trait)	0	0 1	4	0 2
E Mrs Hl *	0 8	0 8	8	1 1
F G K	0	0 1	3 2	0 4
Mrs K (trait)	0 1	<0 1	1 1	0 1
G Mrs B †	0 4	0 25	5 0	1·0
Normal range	0 05–0 5	0–0 4	0 4–4 5	0 03–0 4

* After splenectomy
† Mean values (more than one observation available)

Erythrocyte Life Span in Hereditary Elliptocytosis

It now seems probable that while the life span of elliptocytes is normal or only a little subnormal in the elliptocytosis trait all grades of shortening may be encountered in patients with elliptocytosis and hæmolytic anæmia Some of the more important observations are reviewed below

The earliest reports were based on experiments in which blood containing many elliptocytes was transfused to normal recipients and the elliptocytes subsequently looked for in dried films or wet preparations of the recipients blood Observations of this type suggested that the

Autohæmolysis Selwyn and Dacie (1954) reported some preliminary studies in all except one patient (Case 11 of Dacie *et al* 1953) autohæmolysis at 48 hours (no added glucose) was normal irrespective of whether there was evidence of increased hæmolysis *in vivo* It was noted however that the addition of

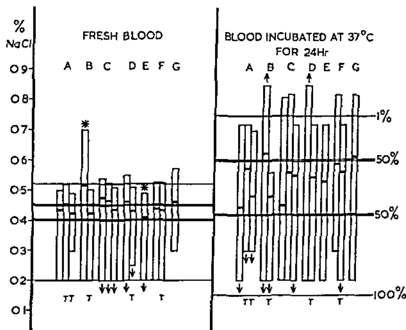


FIG 52 The results of osmotic fragility tests carried out on the *blood of 14 patients with hereditary elliptocytosis*
Left hand chart fresh blood *Right hand chart* the same blood tested after incubation for 24 hours at 37 C
A B C D E F and G refer to different families T represents the elliptocytosis trait the other patients suffered from various degrees of anæmia and increased hæmolysis (see also Table 6)
↑ and ↓ denotes > 1 / or < 100 lysis respectively (see also Fig 40) * After splenectomy

glucose failed in some instances to diminish hæmolysis to the extent expected The author has since studied several further patients with hereditary elliptocytosis and hæmolytic anæmia Four of them had increased osmotic fragility before and after incubation (Fig 52 Families C D and G) and as might be expected this was associated with marked or moderate increases in autohæmolysis The addition of glucose reduced hæmolysis

The erythrocyte osmotic fragility was normal but except for this and the presence of the elliptocytes the hæmatological findings and the patient s symptoms and physical signs were said to be typical of congenital hæmolytic anæmia

Grzegorzewski (1933) described a family in which the erythrocytes of 14 persons were elliptic Six of them were mildly anæmic and gave a history of being slightly jaundiced from time to time their erythrocyte osmotic fragilities were reported to be increased

Mason (1938) described two most interesting examples of hæmolytic anæmia with ovalocytosis (elliptocytosis) The first patient was a white boy of 13 years who had experienced five attacks of anæmia in the preceding 8 years A large proportion (80 %) of his erythrocytes were oval their osmotic fragility was normal His mother was not anæmic but in her blood film there were many oval cells His father s blood was normal but three children of a paternal aunt had died of an undiagnosed severe anæmia It seemed as if the patient s father might have been carrying a latent trait for anæmia which in his son converted a harmless elliptocytic trait into overt hæmolytic anæmia Mason s Case 4 was remarkable in that many of the elliptocytes were deformed and had tail like processes The patient s spleen had been removed 14 years previously this probably had something to do with the morphological peculiarities (see Fig 61 and p 166)

Lambrecht (1938) observed two instances of anæmia with jaundice and decreased osmotic resistance He considered that cases of elliptocytosis might be separated into active (hæmolytic) compensated and latent forms

Giffin and Watkins (1939) studied three patients in one family who suffered from slight to moderate anæmia Elliptocytes and microcytes were present in their blood films Their serum bilirubin concentrations were increased (2 2 to 2 9 mg per 100 ml) and the erythrocyte osmotic fragilities were also slightly increased

Penfold and Lipscomb (1943) described elliptocytosis associated with hereditary hæmorrhagic telangiectasia Five of their patients were jaundiced two had palpable spleens

Holst Larsen s (1947) observations were remarkable especially for the variable intensity of the erythrocyte abnormality in his patients Unmistakable hæmolytic anæmia was present in three branches of a single family the elliptocytes being admixed with and seemingly merging into small microspherocytes and irregularly shaped microcytes in the more anæmic patients

Lendval (1949) briefly described the incidence of severe anæmia in three brothers and sisters The parents were cousins typical elliptocytosis was transmitted from the maternal side on the paternal side 14 brothers and sisters died probably of hæmolytic anæmia This seems to be another example of the effect of the inheritance of traits for congenital anæmia from both sides of a family resulting in severe anæmia in children of the next generation (*cf* Mason 1938)

Dacie and his co workers (1953) described a remarkable instance of severe hæmolytic anæmia in a child who belonged to a family known to carry the elliptocytic trait The child was admitted into hospital when only 10 days old suffering from a rapidly increasing anæmia He was transfused and kept alive for the next 6 months on blood transfusions Splenectomy was then carried out with immediate

survival of the elliptocytes was considerably shorter than normal (Vischer 1938–39 Kirkegaard and Larsen 1942) Later Hedenstedt reported that elliptic cells had an average half life of 13 1 days and that they disappeared from the recipient s circulation in an exponential manner Trinick (1948) who transfused the blood of a healthy non anæmic blood donor 90% of whose erythrocytes were elliptical into a recipient recovering from a recent blood loss found on the other hand using the Ashby method that the donor blood survived normally *i e* 100–110 days

Berlin and Hedenstedt s (1952) report cast doubt on the validity of attempting to deduce the survival of elliptocytes by actually counting the proportion of elliptic to normal cells in the recipient s circulation *Berlin and Hedenstedt found that counts carried out on the same blood* by the Ashby method gave a much longer half life and they concluded that elliptocytes might be transformed into rounded corpuscles in a normal environment This rather unexpected conclusion has not been substantiated by later work Motulsky and his colleagues (1954) repeated Berlin and Hedenstedt s experiment using the blood of three different donors Roughly similar results were obtained by both the visual and Ashby methods In two of the experiments the survival of the transfused elliptocytes was normal that of the corpuscles of the third donor who on other grounds was thought to be suffering from well compensated hæmolysis was impaired (elimination complete in 45 days)

The survival of elliptocytes has more recently been studied in the patients own circulation by the radioactive chromium method Josephs and Avery (1955) reported a ^{51}Cr half time of 16 days in an anæmic child with elliptocytosis while Avery (1956) found the survival to be normal in an infant with elliptocytosis and hæmoglobin C trait and moderately impaired in a sibling with elliptocytosis only who presented other evidence of excess *hæmolysis* McBryde *Hewlett and Weisman (1956)* reported a diminished survival (^{51}Cr half time 18 5 days) in an anæmic negro aged 53 years considered to have hereditary elliptocytosis and Blackburn Jordan Lytle Swan and Tudhope (1958) observed ^{51}Cr half times of 4 5 days and 24 days before and after splenectomy respectively in a man of 67

Data on three of the author s patients whose erythrocyte survival has been measured by the ^{51}Cr method are given in Table 6

Case Reports of Hereditary Elliptocytosis with *Hæmolytic Anæmia*

Some of the more interesting and/or historic reports on hereditary elliptocytosis in which hæmolysis was a conspicuous feature are *summarized* below

One of the first reported cases of overt hæmolytic anæmia associated with elliptocytosis seems to be that of Hijmans van den Bergh (1928)

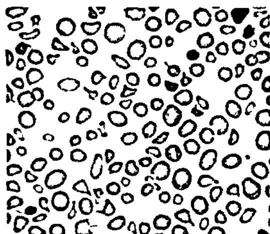


Fig. [illegible]4. Photomicrograph of a blood film of D. H. (Case 11 of Dacie *et al.* 19[illegible]3). Many irregularly shaped spherocytes and less densely staining cell fragments are present. Only a few typical elliptocytes can be seen (but see Fig. [illegible]). [illegible]00.

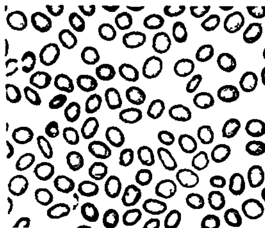


Fig. [illegible]. Photomicrograph of a blood film of Mrs. H., the mother of D. H. (Fig. [illegible]4), who has the elliptocytosis trait. There is a moderate but very definite degree of elliptocytosis. × [illegible]00.

benefit Thereafter erythrocyte regeneration kept pace with hæmolysis (Fig 53) Post splenectomy blood films were remarkable for the marked variation in erythrocyte size and for the presence of numerous *extremely small spherocytic microcytes and fragments of irregular* shape (Fig 54) Erythrocyte osmotic and mechanical fragilities were markedly increased The blood of the child s father was normal but that of his mother and of a brother contained many oval or slightly

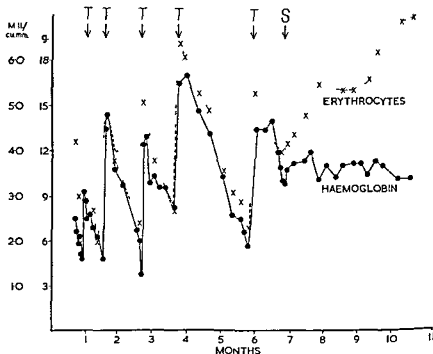


FIG 53 Changes in the erythrocyte count and hæmoglobin concentration of the blood of D Hi (Case 11 of Dacie *et al* 1953) who underwent splenectomy when 6 months old for an unusual type of hereditary elliptocytosis (see also Figs 54 5 and Family B Table 6)
T = transfusion S = splenectomy

elliptic cells (Fig 55) Neither his mother nor brother was anæmic The severe degree of the anæmia of the affected child is unexplained there was no obvious history of anæmia on the father s side of the family

More recent reports include those of Dacie (1954) Motulsky and co-workers (1954) Wilson and Long (1955) Letman (1955) Lipton (1955) Josephs and Avery (1955) Avery (1956) Ducla Soares and Parreira (1958) and Blackburn and co-workers (1958) The erythrocyte life span studies carried out on some of these patients have already been referred to (p 158)

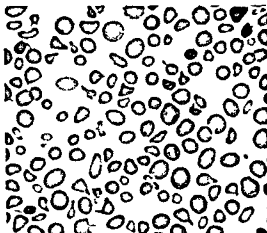


Fig 54 Photomicrograph of a blood film of D H (Case II of Dacie *et al* 19 3) Many irregularly shaped spherocytes and less densely staining cell fragments are present Only a few typical elliptocytes can be seen (but see Fig) × 00

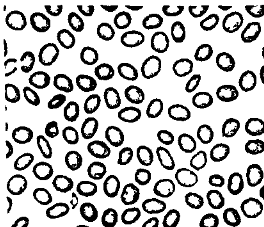


Fig 55 Photomicrograph of a blood film of Mrs H, the mother of D H (Fig 54) who has the elliptocytosis trait There is a moderate but very definite degree of elliptocytosis × 700

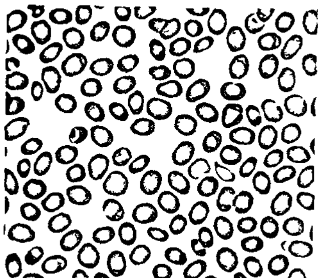


Fig 56 Photomicrograph of a blood film of Mrs Ja (Case of Dacie (1954) see also Family A Table 6) There is a moderate degree of elliptocytosis × 700

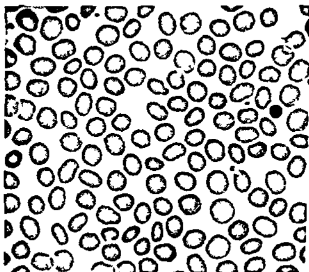


Fig 57 Photomicrograph of a blood film of Mr Lo the father of Mrs Ja who had the elliptocytosis trait Elliptocytosis is slight in degree × 700

The patient described by Dacie (1954) was interesting on several counts first the mode of onset which was a rapidly progressive anæmia appearing as a complication of pneumonia in a woman of 35 and secondly because there was clear evidence of anæmia and hæmolysis but only a moderate degree of elliptocytosis (Table 6 and Fig 56) and normal erthrocyte osmotic fragility and autohæmolysis (Fig 52 and Table 7)

The patient s father and her two sons had the elliptocytosis trait the degree of elliptocytosis was however slight and the erythrocytes were mostly oval rather than elliptical The father s blood was particularly interesting it might not have been recognized as abnormal except for the fact that elliptocytosis was being carefully looked for (Fig 57)

Patients Probably Homozygous for Hereditary Elliptocytosis

As already indicated family studies have suggested that nearly all the patients who have been reported as having hereditary elliptocytosis with hæmolytic anæmia even of a severe grade have been heterozygous for the gene(s) for elliptocytosis Nevertheless at least two probable homozygotes have been described (Wyandt Bancroft and Winship 1941 Lipton 1955)

Wyandt Bancroft and Winship s patient was found amongst a large family of which 86 members had elliptocytosis trait Of these only the suspected homozygote was anæmic or showed any signs of increased hæmolysis The affected patient was a child whose blood contained both elliptocytes and small spherical microcytes The blood of both of his parents contained typical elliptical erythrocytes but neither was anæmic It seems probable therefore that the child inherited a gene for elliptocytosis from both parents and that although a single gene for elliptocytosis produced (in the family) a benign morphological abnormality only the presence of two genes resulted in a severe hæmolytic anæmia

The history of Lipton s patient an infant is also extremely interesting As both patients were first cousins and had the elliptocytosis trait it seemed probable that the infant was homozygous for elliptocytosis It was jaundiced at birth and subsequently became markedly anæmic. After periodic transfusions splenectomy was carried out when the child was 7 months old No transfusions were required thereafter The infant s blood film was remarkable for the microelliptocytes present after splenectomy fragmentation and budding was conspicuous (*cf* Fig 54)

Case Reports Hereditary Elliptocytosis with Hæmolytic Anæmia

Cases 1–3 Brief details of a family in which three members have hereditary elliptocytosis are given below The patients were investigated by the author through the courtesy of Dr Allan Birch They contrast sharply with the patient reported previously (Dacie 1954 Case 5) in that their erythrocytes were morphologically much more abnormal and their osmotic fragilities and rates of autohæmolysis abnormal also

The propositus (B A) was a girl aged 18 who gave a history of 4 months jaundice and tiredness and also of dysmenorrhœa and scanty periods Her younger sister (J.A) aged 14 had also had attacks of

jaundice intermittently since the age of 9 years Examination of B A revealed a slightly jaundiced anæmic young woman whose spleen was palpable 6 cm below the costal margin The blood counts of the two sisters and their mother are recorded in Table 6 (Family C) photomicrographs of their blood films are shown in Figs 58–60 The erythrocyte osmotic fragility of the two girls was slightly but definitely increased (Fig 52) but that of their mother was normal After incubation at 37 C for 24 hours the osmotic fragility of the blood of the two girls fell just outside the normal range but no lysis took place in 0 85% NaCl (Fig 52) the mother's corpuscles behaved normally Autohæmolysis of the girls blood at 48 hours was markedly increased and that of the mother's blood slightly increased (Table 7) The markedly increased autohæmolysis was an unexpected finding in view

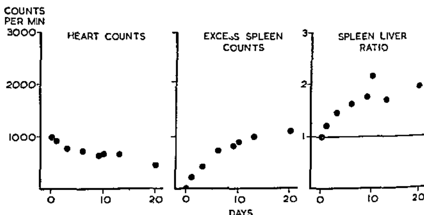


FIG 62 Results of ^{51}Cr erythrocyte survival studies carried out on Case 1 The radioactivity over the heart spleen and liver has been measured by in vivo surface counting The charts show a significant take up of chromium by the spleen and a rising spleen liver ratio (*cf* Fig 32)

of the fact that the increase in osmotic fragility following incubation was only slightly greater than normal (*cf* hereditary spherocytosis p 102)

The survival of the erythrocytes of B A were measured in her own circulation using ^{51}Cr The mean cell life span was calculated to be 14 days Surface counting demonstrated a marked take up of chromium by the spleen (Fig 62) similar to that seen in hereditary spherocytosis

The hæmatological data on several other patients (with hæmolytic anæmia and clinical evidence of increased hæmolysis) investigated by the author are summarized in Tables 6 and 7 and in Fig 52 By way of contrast the data on relatives of the propositus (with elliptocytosis but without clinical evidence of excess hæmolysis) have been included Photomicrographs of the blood films of some of these patients are illustrated in Figs 61 and 63–66 They make a contrasted series and illustrate the variation in erythrocyte morphology between families Figs 55 and 66 show that subjects with elliptocytosis trait tend to have



Fig 8 Photomicrograph of a blood film of B A (Case 1) There is a moderately severe degree of elliptocytosis and a tendency to poikilocytosis. A few cell fragments are also seen. ×1200

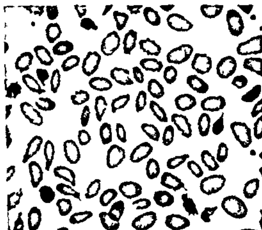


Fig 9 Photomicrograph of a blood film of J A (Case 2) This is similar to that of her sister (Fig 8) except for slightly more microcytosis and crenation ×1200

jaundice intermittently since the age of 9 years Examination of B A revealed a slightly jaundiced anæmic young woman whose spleen was palpable 6 cm below the costal margin The blood counts of the two sisters and their mother are recorded in Table 6 (Family C) photomicrographs of their blood films are shown in Figs 58–60 The erythrocyte osmotic fragility of the two girls was slightly but definitely increased (Fig 52) but that of their mother was normal After incubation at 37 C for 24 hours the osmotic fragility of the blood of the two girls fell just outside the normal range but no lysis took place in 0 85% NaCl (Fig 52) the mother s corpuscles behaved normally Autohæmolysis of the girls blood at 48 hours was markedly increased and that of the mother s blood slightly increased (Table 7) The markedly increased autohæmolysis was an unexpected finding in view

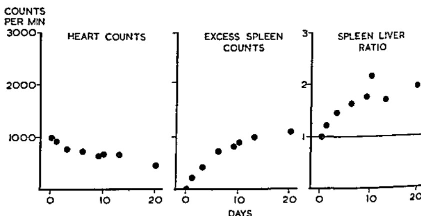


FIG 62 Results of ^{51}Cr erythrocyte survival studies carried out on Case 1 The radioactivity over the heart spleen and liver has been measured by in vivo surface counting The charts show a significant take up of chromium by the spleen and a rising spleen liver ratio (*cf* Fig 32)

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less abnormal blood films than have their relatives with elliptocytosis and hæmolytic anæmia

Classification and Pathogenesis of Hereditary Elliptocytosis with Hæmolytic Anæmia

As already indicated it is now generally agreed that hereditary elliptocytosis is in the majority of instances a harmless trait (Mason 1938 Kirkegaard and Larsen 1940 Penfold and Lipscomb 1943 Guasch and Raichs 1949 Motulsky *et al* 1954 etc) How is it then that in a minority of cases a harmless trait in which excessive hæmolysis is absent or minimal and easily compensated becomes converted into an active uncompensated hæmolytic anæmia?

The possibility that excessive hæmolysis only occurs when the gene for elliptocytosis is present in the homozygous form is not supported by the facts Only in the reports of Wyandt Bancroft and Winship (1941) and Lipton (1955) have both parents of a severely affected child been shown to be carriers of the elliptocytosis trait It seems instead that the expressivity of the gene or genes for elliptocytosis may be markedly modified in other ways than by the gain of an additional similar gene and that varying grades of increased hæmolysis are the result of this modification As already mentioned it is remarkable that although overt hæmolytic anæmia is unusual when present several members of a family may be similarly affected (see Case Reports p 159) This suggests that there are at least two types of hereditary elliptocytosis a benign typical form not associated with hæmolytic anæmia (unless in the homozygous state (Wyandt Bancroft and Winship 1941)) and a rarer type or types often associated with different grades of hæmolytic anæmia The morphological and other hæmatological differences between patients with active hæmolytic anæmia which have already been mentioned are certainly in favour of the hypothesis that more than one type of hereditary elliptocytic hæmolytic anæmia exist but until the fundamental defect of the erythrocytes can be defined it may prove impossible to determine whether the differences are quantitative or qualitative

It is not possible to correlate the degree of elliptocytosis with the presence or absence of hæmolysis On the one hand a marked degree of elliptocytosis is not necessarily accompanied by signs of hæmolysis and anæmia on the other hand overt hæmolytic anæmia as in Case 5 of Dacie (1954) may be associated with only a moderate degree of elliptocytosis However as has already



Fig 60 Photomicrograph of a blood film of Mrs A (Case 3) The appearances are similar to those of her daughters films (Figs 58 and 3) but the general picture is slightly less abnormal × 00

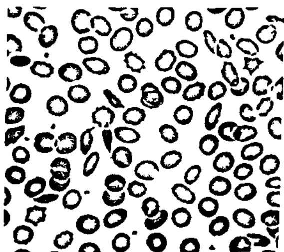


Fig 61 Photomicrograph of a blood film of Mrs III (Family I Table 6) After splenectomy A number of elongated (oat shaped) cells are present and there is also a moderate degree of crenation × 00

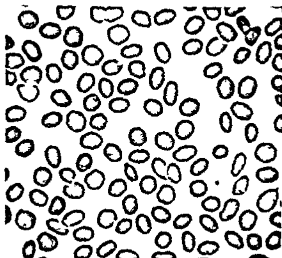


Fig. 63. Ph ton ..r.graph of blood film of I. Hw (Family D Table 8). Oval ... present and no cell fragment ... 00

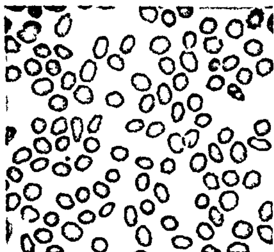


Fig. 64. Ph t micrograph of a blood film of Mrs B (Family C Table 6) ... but there ... of ... 00

been mentioned within a single family the blood film of a subject with the elliptocytosis trait is usually less abnormal than that of a relative who presents with signs of hæmolysis Furthermore if bizarre shaped cells are present and if there are many round or elliptic spherocyte a greater or lesser degree of hæmolysis can be expected

It does not appear to be likely that the presence of spherocytes (and increased osmotic fragility) necessarily indicates an admixture with the hereditary spherocytosis trait It seems more probable that spherocytosis and microcytosis are one result of an increased expressivity of the gene for elliptocytosis This is well shown by Holst Larsen s (1947) patients by Case 11 of Dacie and co workers (1953) and by the patients of Wyandt Bancroft and Winship (1941) and Lipton (1955) who were probably homozygous for elliptocytosis However as already mentioned an increased rate of hæmolysis is not necessarily associated with microspherocytosis and increased osmotic fragility

Possible Biochemical Abnormalities in Elliptocytosis

There seems little reason to doubt that the abnormality in shape is not the sole erythrocyte abnormality in hereditary elliptocytosis In patients in whom there is increased hæmolysis at least it seems likely that the erythrocytes have a biochemical defect analogous to that of hereditary spherocytosis Unfortunately however the hypothetical abnormality or abnormalities have as yet not been defined Selwyn and Dacie (1954) studied two patients with hæmolytic anæmia as well as two carriers of the elliptocytosis trait The erythrocytes of one of the patients with hæmolytic anæmia had an abnormally low potassium content and although autohæmolysis proceeded at the normal rate in three of the patients the addition of glucose was less effective in reducing hæmolysis than with normal blood and most instances of hereditary spherocytosis Table 7 shows that the effect of glucose varied from family to family In Family C the rapid autohæmolysis and the effect of glucose on it were indistinguishable from that in typical hereditary spherocytosis

Role of the Spleen The favourable results of splenectomy (see p 166) suggest that some types of elliptocytes at least are peculiarly sensitive to the hæmolytic action of the spleen Sections of spleens removed at operation show congestion of the pulp and it seems probable that the mechanism of hæmolysis is analogous to that in hereditary spherocytosis It does not seem likely that the sequestration of elliptocytes in the spleen can be entirely explained on their abnormal shape in view of the fact that a marked degree of elliptocytosis is apparently compatible with a normal erythrocyte life span The sequestration must be due to

some more subtle ? biochemical abnormality the nature of which is as yet unknown

Differential Diagnosis

In most cases the recognition of hereditary elliptocytosis presents no difficulties The blood film is usually characteristic and a family study clinches the diagnosis Oval cells and elliptocytes are of course found in many blood diseases and may be particularly numerous in iron deficiency anæmia and myelosclerosis but in contradistinction to hereditary elliptocytosis it is most unusual to find as many as 80–90% of the cells affected and acquired elliptocytosis is usually also associated with a much greater degree of poikilocytosis than is the congenital disease

The degree of shape change may however be mild in hereditary elliptocytosis (see Figs 56 and 63) and if a minor degree of elliptocytosis is associated with hæmolysis the differentiation from hereditary non spherocytic hæmolytic anæmia can be difficult A family study may prove helpful in such cases The distinction is important because of the benefit to be expected from splenectomy in true hereditary elliptocytosis

Some cases of thalassæmia may be confused with hereditary elliptocytosis The presence of more poikilocytes target cells and obvious hypochromia are points in favour of thalassæmia Here again a family study helps Another source of confusion is iron deficiency superimposed on hereditary elliptocytosis (Josephs and Avery 1955 Avery 1956) In such cases the elliptocytosis persists after adequate iron therapy

Treatment of Hereditary Elliptocytosis with Hæmolytic Anæmia

Blood Transfusion There is practically no information on the survival of normal erythrocytes after transfusion to patients suffering from hereditary elliptocytosis but what evidence there is suggests that normal corpuscles survive for the normal length of time In Case 11 of Dacie and co workers (1953) the survival of transfused blood was proved to be normal the child being literally kept alive by transfusion The same was true of Lipton s (1955) patient who was probably homozygous for elliptocytosis Transfusion can therefore be confidently recommended as a *palliative measure in the severely anæmic patient*

Splenectomy There are by now a good many reports in the literature on the effects of splenectomy in hereditary elliptocytosis

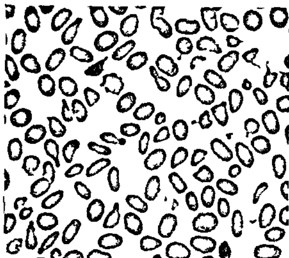


Fig 65 Photomicrograph of a blood film of R Kr (Family II Table 6) Elliptocytosis is moderately severe in degree and poikilocytes micro spherocytes and cell fragments are all quite conspicuous × 700

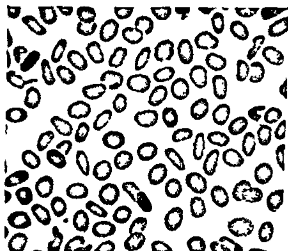


Fig 66 Photomicrograph of a blood film of Mr Kr (Family II Table 6) who has the elliptocytic trait This blood film is considerably less abnormal than that of her son (Fig 65) who has overt haemolytic anaemia × 700

and Crosby (1957) illustrated (their Fig 7) the histological appearance of the spleen of one case of hereditary ovalocytosis and remarked on its similarity to that of hereditary spherocytosis The spleen pulp of Case 11 of Dacie and co-workers (1953) was moderately congested with blood the sinuses on the other hand were relatively empty Blackburn and his colleagues (1958) described the spleen of their patient which weighed 537 g as having moderate congestion of the pulp with marked prominence of the reticulo-endothelial cells which lined the pulp spaces They also found evidence of erythrophagocytosis

These findings support the idea that in many patients at least with hereditary elliptocytosis and hæmolytic anæmia there is an unusual degree of erythrocyte sequestration in the spleen pulp As in hereditary spherocytosis this probably explains the beneficial result of splenectomy

Hereditary Elliptocytosis in Association with other Traits

It has already been mentioned that microspherocytosis and increased osmotic fragility may be observed in some patients suffering from hereditary elliptocytosis with hæmolytic anæmia In none of the case reports so far recorded does there seem however to have been conclusive evidence based on family studies of the presence of the trait of hereditary spherocytosis in addition to that of hereditary elliptocytosis The early reports of the association of elliptocytosis with the sickle cell trait (Pollock and Dameshek 1934 Fadem 1949) are likewise inconclusive

Recently however Vandepitte and Louis (1955) have reported two families from the Congo in which both traits were conclusively demonstrated The two genes segregated independently

The propositus of Vandepitte and Louis s first family probably had homozygous sickle-cell disease plus elliptocytosis the father had Hb-S trait and the mother Hb S trait plus markedly elliptical erythrocytes—she was slightly anæmic The elliptical cells and bacteriocytes (very elongated cells) sickled as readily as did round cells Of other children in the family one was normal another had Hb S trait plus elliptocytosis trait and another elliptocytosis trait alone The mother s sister had elliptocytosis trait The propositus of the second family had Hb S trait and elliptocytosis and a moderate hæmolytic anæmia

Vandepitte and Louis calculated that the combination of Hb S and elliptocytosis traits should not be uncommon in the Congo There is a 25 incidence of Hb-S trait and they suggested that the frequency of the elliptocytosis trait is 1 in 250 the combination should therefore have an incidence of 1 in 1 000 Vandepitte and Louis concluded that the Hb-S trait does not accentuate the clinical effects of a single gene for elliptocytosis and *vice versa*

The combination of elliptocytosis with Hb-C has also been conclusively proved by family studies (Avery 1956) The father of the propositus had the elliptocytosis trait and the mother Hb C trait Two of their children had both traits in combination They

with hæmolysis Most of the patients seem to have benefited but unfortunately in most instances only scanty details are given

Hijmans van den Bergh (1928) reported that the jaundice of his patient receded Mason (1938) recorded the blood count of a patient (his Case 4) splenectomized 14 years previously as 4 230 000 erythro cytes per cu mm and 72% hæmoglobin

Giffin and Watkins (1939) described good results in two cases after splenectomy with improvement in anæmia reduction in jaundice and less evidence of regeneration than before operation Holst Larsen (1947) mentioned the effect of splenectomy in two severely affected patients Although a substantial rise in hæmoglobin and loss of jaundice followed the operation the degree of elliptocytosis and spherocytosis was unaffected

Lendval (1949) reported improvement after splenectomy and stated that whereas during a hæmolytic phase (before splenectomy) erythrocyte thickness was increased after splenectomy only normal elliptocytes were present Another successful result was reported by Harrier and his colleagues (1952) Wilson and Long (1953 1955) reported the presence of hæmolytic anæmia and ovalocytosis in two elderly patients (a brother and his sister) Splenectomy carried out on one of them resulted in cure of the anæmia and leucopenia After operation an increased number of elliptical cells triangular cells and spherocytic microcytes was found in blood films

The patient described as Case 11 by Dacie and co workers (1953) derived striking benefit from splenectomy before operation the child s life depended on transfusion after operation erythrocyte formation more than kept pace with destruction (Fig 53) Whether patients whose erythrocytes have an increased osmotic fragility do better after splenectomy than those with normal fragility remains to be seen

Lipton s (1955) patient an infant thought to be a homozygote similarly benefited strikingly from splenectomy Letman s (1955) patient an adult aged 41 was well for many years subsequently The adult patient of Blackburn and his co workers (1958) also benefited from splenectomy one year after operation his hæmoglobin was 16 g per 100 ml

Blood Picture after Splenectomy

A striking feature is the increased number of micro elliptocytes and/ or bizarre shaped cells which circulate (Lipton 1955 Letman 1955 Wilson and Long 1955 Figs 54 and 61) Fragmentation appears to be taking place It seems likely that the bizarre blood picture which results from splenectomy is due to the increased life span of the erythrocytes There is probably a tendency for time expired elliptocytes to undergo fragmentation and for the fragments to circulate for an unduly long period as the result of the removal of the filtering action of the spleen (see Wilson and Long 1955 Lipton 1955)

Splenic Histology Few reports are available Lipton (1955) described the spleen (65 g) of his patient an infant as engorged with blood and as having congested sinusoids The spleen of Wilson and Long s (1955) patient weighed 640 g its pulp was congested and dilated and there was some reticular hyperplasia fibrosis and siderosis Rappaport

GOODALL, H B HENDRY D W W LAWLER S D and STEPHEN S A (1953) Data on linkage in man elliptocytosis and blood groups II Family 3 *Ann Eugen (Lond)* 17 272
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were anæmic in one however the erythrocyte life span was shown to be normal and the other responded to treatment with iron

A few interesting examples are on record of other types of blood disease occurring in association with the trait for hereditary elliptocytosis the patient of Bang and Georg (1947) for instance may have suffered from paroxysmal nocturnal hæmoglobinuria and that of Druez (1952) from an acquired hæmolytic anæmia of the auto immune type

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CHAPTER 4

THE CONGENITAL HÆMOLYTIC ANÆMIAS

3 HEREDITARY NON SPHEROCYTIC HÆMOLYTIC ANÆMIA "ATYPICAL AND UNCLASSIFIED TYPES "ERYTHROPOIETIC PORPHYRIA" AND CONGENITAL HEINZ-BODY ANÆMIA

In this chapter will be considered certain types of congenital hæmolytic anæmia which differ in several important respects from hereditary spherocytosis but with which they are nevertheless often confused There are several such disorders and in most of them splenectomy is not followed by permanent clinical cure Morphologically with the exception of some atypical cases and the rare congenital Heinz body anæmia the blood picture does not as a rule present any striking features The red cells tend to be slightly macrocytic most are round in contour but sometimes elliptocytes and poikilocytes are present in moderate numbers and punctuate basophilia may be conspicuous Spherocytes are *characteristically not present or only present in very small* numbers and osmotic fragility is typically not increased

The disorders are rare but probably not as rare as the relatively few reports in the literature would suggest Hereditary non spherocytic hæmolytic anæmia, of which there are at least two varieties (Types I and II (Selwyn and Dacie 1954)) is most frequently met with This will be discussed first then some atypical unclassified types and finally congenital hæmolytic anæmia associated with porphyria (erythropoietic porphyria) and congenital Heinz body anæmia.

Hereditary Non Spherocytic Hæmolytic Anæmia

Case Reports in the Literature

Baty (1930) described a severe hæmolytic anæmia in a boy aged 3 years which seems in retrospect likely to be an example of hereditary non spherocytic hæmolytic anæmia although the family history was negative Osmotic fragility was normal and splenectomy did not result in clinical cure Jaundice and anæmia persisted and reticulocyte counts between 30 and 90 / were recorded after splenectomy The spleen was deep red in colour histological sections showed empty sinuses an increase in fibrosis of the trabeculæ and extramedullary hæmopoiesis

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During and shortly after this hæmolytic episode the survival of transfused normal corpuscles was impaired Later transfused normal corpuscles survived normally

Another patient suffering from an apparently congenital hæmolytic anæmia was described by Feinberg and Watson (1951) The patient was a negro his eight brothers and sisters and his two children appeared to be unaffected His anæmia was normochromic and slightly macrocytic and a striking feature of his blood film was the large number of stippled cells present Osmotic fragility before and after incubation at 37 C was normal and tests for sickling were repeatedly negative A splenic aspiration was carried out smears showed fewer stippled cells than in the peripheral blood Feinberg and Watson concluded that the spleen was either destroying the stippled cells or sifting out the inclusions from the stippled cells This disorder seems to be very similar to that affecting the second of Haden s families

Holliday (1953) described a family in which at least four members suffered from a non spherocytic hæmolytic anæmia Basophilic stippling of the erythrocytes was conspicuous in three of the patients One patient was studied in considerable detail mechanical fragility was found to be slightly increased and the patient s erythrocytes were reported to undergo more rapid autohæmolysis both at 4 C and at 37 C when suspended in sterile isotonic saline than did normal corpuscles In plasma however the rate of autohæmolysis was normal

Lipton Grossman and Richmond (1953) described the clinical and hæmatological data in two sisters who probably suffered from a hereditary non spherocytic hæmolytic anæmia The early results of splenectomy carried out on the older child were encouraging although anæmic he managed to compensate for hæmolysis after operation without transfusions being necessary

Dacie Mollison Richardson Selwyn and Shapiro (1953) described four patients with congenital non spherocytic hæmolytic anæmia belonging to different families all had undergone splenectomy without their anæmia being alleviated

Case 1 a woman aged 29 years had had her spleen removed in early childhood Her erythrocytes (after splenectomy) were mostly macrocytes rounded in contour and nearly all contained Pappenheimer bodies (Fig 69) Osmotic fragility was slightly diminished Hæmolysis *in vivo* was evidently greatly accelerated for her reticulocyte count constantly exceeded 60 % The normal survival of transfused normal corpuscles is shown in Fig 67 A notable feature was that her blood underwent spontaneous hæmolysis *in vitro* at more than ten times the normal rate (see p 182 Case No 14)

Case 2 was a boy aged 7 years whose spleen had been removed 6 months previously without substantially influencing the course of his disease His erythrocytes (after splenectomy) were mostly rounded in contour and slightly macrocytic Osmotic fragility was slightly increased

Case 3 a boy aged 17 had undergone splenectomy when 14 years of age without the operation influencing the course of his disease Before splenectomy many of his erythrocytes were macrocytes some were slightly oval in shape In addition occasional pear shaped poikilocytes and small contracted corpuscles were present After

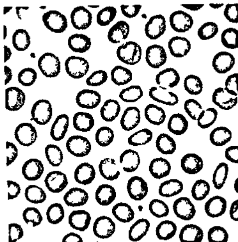
Thompson (1939) referred briefly to three families suffering from hereditary hæmolytic anæmia *with normal erythrocyte osmotic* fragilities He mentioned that splenectomy had been carried out in several of the patients without improvement

Haden (1947) described two families one American and the other Hungarian affected with a new type of hereditary hæmolytic jaundice In the first family three members of two generations were affected in the second family four members of three generations In both families the anæmia was macrocytic in type osmotic fragilities were normal and there was no spherocytosis Splenectomy was carried out on one patient but this did not alter the course of the disease Haden s description indicates that although both families were affected by a non spherocytic hæmolytic anæmia there were important differences in the type of anæmia in the two families A notable feature of the anæmia affecting the first family was the rapidity of autohæmolysis *in vitro* in the second family the most notable feature was the striking punctate basophilia of the erythrocytes

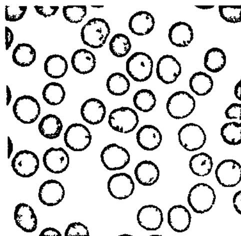
Crosby s (1950) report dealt with a large American family of mixed English and French antecedents in which a relatively mild chronic normocytic hæmolytic anæmia was found in seven (possibly in nine) out of 36 members Brachyphalangia was also found but this was not necessarily associated with anæmia One patient was investigated in detail *Splenectomy was carried out but without benefit to his anæmia* Morphologically his erythrocytes were biconcave discs occasional cells were oval or tailed a very few were spherocytes or target cells The erythrocyte mechanical fragility was normal but autohæmolysis on incubation was accelerated An interesting additional abnormality was the presence of porphobilinogen in his urine on several occasions —he had however no definite symptoms or clinical signs of porphyria It is also interesting to note that all the anæmic members of the family belonged to blood group A

Kaplan and Zuelzer (1950a) found a hæmolytic anæmia in three out of the six children of a family of French Canadian extraction Each child suffered from a moderately severe and slightly macrocytic anæmia There were no target cells or spherocytes but about half of the corpuscles were slightly or moderately oval The osmotic and mechanical fragilities were normal Splenectomy was not carried out It is possible that the children inherited the disease from their mother as about 15% of her *corpuscles were slightly oval and she was mildly anæmic* However the mother s reticulocyte count was within the normal range As the degree of ovality of the patients erythrocytes was far less than in typical hereditary elliptocytosis Kaplan and Zuelzer did not consider that there was any relationship between the anæmia from which the patients were suffering and hereditary elliptocytosis

Kaplan and Zuelzer (1950b) in another publication reported observations on two further children of Italian and American origin who also were affected with non spherocytic hæmolytic anæmia Their anæmia was normocytic in type and there were occasional microspherocytes the osmotic resistance was however increased Splenectomy resulted *in slight improvement only The elder of the two children developed* a transient acute hæmolytic episode with marked microspherocytosis and hæmoglobinuria apparently due to the formation of auto antibodies



I n C8   I I otomicro rnph of a l lood f lm c f a patient suffcrin from hereditary non pl rocytic hæmolytic an m a (Type I) (Case 5 of D ie *et al* 1953) (Case   Tal le 0)   Bef re sp l nectomy   Some of tl e erytl ocytes re ov l or ell ptical in shape   × 700



F c (J   I I otomicrograpl of a blood film of a patient sufferin from l reditary n n pl erocytic hæmolyt c a æmia (Type II) (Ca e 1 of D ie *et al* 1953) (Case 14 Tabl 0)   Splen ctomy l ad been c ried out mo   tl n   years p   io isly

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splenectomy target cells were conspicuous (Fig 75) Osmotic fragility was normal before splenectomy and slightly diminished afterwards He was admitted into hospital in February 1957 when aged 2- for reassessment His hæmoglobin was stable at about 7 4 g per 100 ml the reticulocyte count varied between 4 5 and 8 7% The platelet count was 1 040 000 per cu mm The mean erythrocyte life span was estimated to be 6 5 days (^{51}Cr method) Unfortunately during this admission he died suddenly from a pulmonary embolism Post mortem

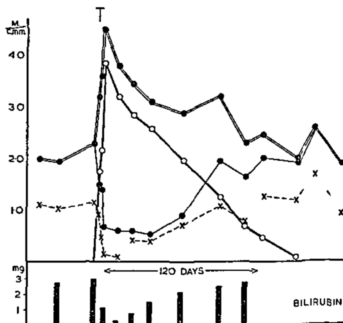


FIG 67 Hæmatological changes following a large blood transfusion given to a patient suffering from hereditary non spherocytic hæmolytic anæmia (Type II) (Case 1 of Dacie *et al* 1953)
●═══● = the total erythrocyte count o——o = the count of donor s erythrocytes ●——● = the recipient s count x----x = the absolute reticulocyte count

examination showed in addition to massive embolization of the main pulmonary arteries a hyperplastic bone marrow enlargement of the liver (2 460 g) and some enlargement of the lymph nodes No splenunculi were found Histological examination showed marked hæmosiderosis of the liver and certain lymph nodes The Kupffer cells of the liver were not particularly conspicuous and most of the iron was in the parenchyma cells Heavy deposits of hæmosiderin were also demonstrable in the cells lining the 2nd convoluted tubules of the kidney The bone marrow was patchily hyperplastic and erythropoiesis

was conspicuous Erythrophagocytosis in abnormal amounts was not seen in any of the tissues examined

Case 4 a girl aged 13 years had had her spleen removed 6 years previously Hæmolysis was still proceeding at a rapid rate Her erythrocytes (after splenectomy) were mostly macrocytes with a round contour containing conspicuous Pappenheimer bodies This case seemed to be almost identical with Case 1 More recently 13 years after splenectomy this patient s mean erythrocyte life span was estimated by the ^{51}Cr method to be only about 5 days—the reticulocyte count at this time was approximately 70 %

Dacie and co-workers (1953) also described a fifth patient (Case 5) suffering from a congenital non spherocytic hæmolytic anæmia His spleen had not been removed at the time of their report He was a boy aged 15 years only moderately anæmic but always visibly jaundiced with a serum bilirubin level usually in the region of 4–5 mg per 100 ml His erythrocytes were slightly macrocytic with a definite tendency to elliptocytosis (Fig 68) His mother appeared to have the trait in a very mild form

Further details of this patient can now be given as clinically obvious jaundice was continuously present it was thought advisable to carry out splenectomy even though the chances of any marked improvement seemed remote The spleen was moderately enlarged it was found to weigh 260 g when allowed to empty itself of blood (about twice the normal weight for the patient s age) Histological examination showed less congestion with blood than in hereditary spherocytosis (Fig 76) The Malpighian bodies were normal in size the pulp cords were unusually prominent and contained moderate numbers of erythrocytes The littoral cells of the sinuses were conspicuous and iron containing pigment was present in moderate amounts

No substantial benefit resulted from removal of the spleen The hæmoglobin level ranged between 11 3 and 12 7 g per 100 ml during the first year after operation and the reticulocyte count varied between 4 7 and 7 4 % as compared with pre operative hæmoglobin levels and reticulocyte counts of 11 0–11 5 g and 4–6% respectively On the other hand the serum bilirubin level was slightly lower averaging 2 7 mg per 100 ml between 3 and 12 months after operation compared with a pre operative average figure of 4 4 mg per 100 ml When last examined 4 years after splenectomy the hæmoglobin was 13 1 g per 100 ml the reticulocyte count was 6 0 % and serum bilirubin 1 5 mg per 100 ml He reported that he felt fit

More recently a fair number of additional cases have been reported The more interesting of these are referred to below

Nelson s (1954) patient was a girl aged 7 years who had been mildly jaundiced since birth She had a mongoloid facies and an X ray of the skull showed thickening of the diploe and vertical striæ The spleen was removed it was cellular due to an increase in reticulum cells but not congested with blood The child appeared to derive some benefit from splenectomy the recorded hæmoglobin levels before operation being 2 8–7 4 g per 100 ml and during the first 4 years after operation 8 4–10 1 g per 100 ml Cortisone in large doses (150 mg daily 1 7 g in 16 days) appeared to raise the reticulocyte leucocyte and platelet counts but did not alter the erythrocyte count or bilirubin level

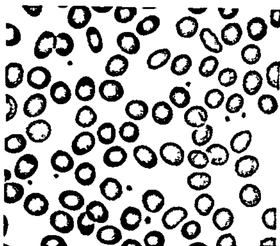


FIG 70 Photomicrograph of a blood film of a patient suffering from hereditary non spherocytic hæmolytic anæmia (Type I) (Case 10 Table 9) Some of the cells are oval or elliptical in shape × 700

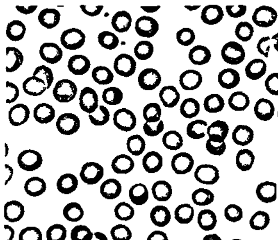


FIG 71 Photomicrograph of a blood film of a patient suffering from hereditary non spherocytic hæmolytic anæmia (Type I) (Case 9 Table 9) Almost all the erythrocytes are round in contour × 700

studies on a French Canadian family The propositi were two sisters They underwent splenectomy when aged 4 years and 2½ years respectively and both appeared to derive some benefit In all 134 members of the family were examined Of four siblings of the patients one had splenomegaly and a mild hæmolytic anæmia and three lesser degrees of splenomegaly Their mother and five siblings (out of nine examined) also had palpable spleens but were not anæmic Smiley and his co-workers concluded that the anæmia was inherited as a Mendelian dominant but that the gene had variable penetrance and was on the whole of low expressivity

The two children described by Brafield and Reef (1957) had been anæmic and jaundiced since birth Osmotic fragility was normal and autohæmolysis studies suggested that they were Type I cases (see later) The family history was negative but the father (who was not anæmic and had a normal reticulocyte count) had a serum bilirubin level of 1 2-1 5 mg per 100 ml The erythrocytes of a paternal aunt too were thought to give an abnormal result in an autohæmolysis test Splenectomy produced partial remission in both children (see p 180)

Pinkerton (1957) described two families with different types of hereditary non spherocytic hæmolytic anæmia In the first family there were at least five affected members in three generations they appear to be suffering from Type I non spherocytic hæmolytic anæmia The second family consisted of two male siblings only Both had a mild anæmia characterized by marked anisopoikilocytosis of the erythrocytes and the presence of fragmented forms The reticulocyte counts were relatively low (maximum 4 5 %) Autohæmolysis proceeded at the normal rate but neither the addition of glucose nor adenosine reduced this to the normal extent Pinkerton concluded that dyshæmopoiesis as well as hæmolysis were factors in the pathogenesis of the anæmia affecting his second family

de Gruchy Crawford and Morton (1958) have more recently reported on four cases though to be Type II non-spherocytic hæmolytic anæmia Three of the patients were known to be anæmic as infants All were submitted to splenectomy one was thought to be slightly improved thereby Post-operatively their reticulocyte counts ranged from 30 to 60 % After incubation the osmotic fragility was increased to an abnormal extent the rate of autohæmolysis was also increased and this was not diminished by the addition of glucose

Other cases described in recent years include those of Blaser Cretin and Stampfli (1956) Gasser (1958) and Kahler Sundermann and S hubothe (1958)

The author has studied eleven further patients with hereditary non spherocytic hæmolytic anæmia (eight families) in addition to the five patients described by Dacie and co workers (1953) One patient was reported as Case 6 by Dacie (1954) The clinical histories of the others are not particularly noteworthy the hæmatological data are considered below and in Tables 8 and 9

As has been mentioned there is reason to believe that there are at least two types of hereditary non spherocytic hæmolytic

The case reported by Bruton Crosby and Motulsky (1954) was an infant At birth severe hæmolytic disease of the newborn seemed the probable diagnosis (hæmoglobin 6 9 g per 100 ml) However this was not confirmed serologically and the anæmia returned after transfusion No abnormal hæmoglobins were demonstrated by electrophoresis Splenectomy at 10 months appeared to be of no value The child s mother was subsequently found to have a mild non spherocytic hæmolytic anæmia with hæmoglobin 11 2 g per 100 ml and 6 5% reticulocytes Motulsky Crosby and Rappaport (1954) described four cases (including the infant and his mother already reported by Bruton Crosby and Motulsky (1954)) and reviewed the literature

Hennemann (1955) described a young woman aged 20 years who had been anæmic since infancy Splenectomy was carried out when she was 10 years of age but apparently without significant benefit She had a mongoloid facies and X ray of her skull showed tower skull and vertical striæ Her erythrocytes were rounded macrocytes and osmotic fragility normal with fresh blood became markedly increased on incubation Spontaneous hæmolysis was noted after 24 hours at 37 C The reticulocyte count was very high ranging from 25 to 97% A sister died aged 8 months of a blood disease Hennemann s case seems identical with Cases 1 and 4 of Dacie and his colleagues (1953) *i e* it appears to be an example of the rare Type II hereditary non spherocytic hæmolytic anæmia (see p 190)

Saint and Hunt (1955) described a 34 year old woman who had undergone splenectomy without apparent benefit when 2 years of age When she was 15 years old cholecystectomy for pigment gall stones was carried out She died of cardiac failure and hæmochromatosis however as she had received not more than 11 litres of blood in her lifetime Saint and Hunt considered that this factor alone would not explain the very large amount of iron throughout her tissues Increased alimentary absorption possibly as the result of the chronic anæmia appeared to have taken place According to de Gruchy (1958) this patient also was suffering from the Type II disease

Kravit Smith Marvin Read and Good (1956) described two infants both of whom became severely anæmic soon after birth In each case family studies were negative The survival of normal erythrocytes as estimated by the ^{51}Cr method was subnormal in both children but not as short as the children s erythrocytes in their own circulations A laparotomy was carried out in one of the children who had undergone splenectomy at 9 months and an accessory spleen weighing 117 g was removed However no benefit resulted although the diminished survival of normal transfused cells had suggested that an extracorpuscular factor might have been contributing to the hæmolysis This child also received large doses of cortisone and ACTH sufficient to produce Cushing s syndrome without benefit to her anæmia

Horsfall s (1956) patient a boy aged 6 years was one of three affected children His history is remarkable for the development of an aplastic crisis with the hæmoglobin falling to 3 3 g per 100 ml and the reticulocyte count to 0 1% Splenectomy had been carried out at 7 months of age Recovery followed transfusion According to de Gruchy (1958) this is also a Type II case

Smiley Dempsey Villeneuve and Campbell (1956) described extensive

anæmia dating back to early infancy Severe hæmolytic disease of the newborn is sometimes simulated (see Kaplan and Zuelzer 1950b Bruton Crosby and Motulsky 1954 Gasser 1958)

Associated Abnormalities Brachyphalangia was reported by Crosby (1950) in the family he studied but this was not necessarily asssociated with the presence of hæmolytic anæmia A mongoloid facies and thickening of the cranial bones with widening of the diploe and perhaps vertical striæ on X ray examination of the skull seem not to be uncommon (*e g* Kaplan and Zuelzer 1950a Nelson 1954 Hennemann 1955) The changes appear similar to those seen in Mediterranean anæmia and presumably have a similar cause (see p 218)

Symptoms As mentioned earlier anæmia and jaundice usually date from birth and in the worst affected cases the infant may appear to be suffering from hæmolytic disease of the newborn The spleen is often but not invariably palpable Pigments gall stones may occur (Saint and Hunt 1955 de Gruchy 1958)

The degree of hæmolysis ranges from a severity which leads to severe anæmia necessitating repeated transfusions to a degree easily compensated for by the patient The clinical grade of jaundice varies too in some patients (*e g* Case 5 of Dacie *et al* 1953) it may be the dominant symptom As in hereditary spherocytosis most patients tend to stabilize their hæmoglobin concentrations at a particular personal level—that of Case 1 of Dacie and co workers (1953) remained at 7–8 g per 100 ml almost unchanged for more than 10 years despite a very rapid rate of hæmolysis and a reticulocyte count of 50–70% Other patients suffer from occasional episodes of more serious anæmia (minor crises) At least one patient who had undergone splenectomy (Horsfall 1956) suffered from an aplastic crisis

Blood Picture in Hereditary Non Spherocytic Hæmolytic Anæmia

Most of the erythrocytes are rounded macrocytes and there is slight to moderate anisocytosis In the two Type II cases investigated by the author the erythrocytes were conspicuously rounded (Fig 69) in Type I cases a minority of the cells may be distinctly oval or elliptic (Figs 68 and 70 *cf* Fig 71) but the degree of elliptocytosis and the frequency of markedly elliptical cells are much less than in typical hereditary elliptocytosis An occasional tailed poikilocyte may be present but microcytes and cell fragments are usually absent Spherocytic cells are also typically absent but they may be present in very

anæmia Type I the commonest type—*possibly not homogeneous* and Type II the rarer type of which Cases 1 and 4 of Dacie and co workers (1953) were the prototypes and of which the patients of Baty (1930) Hennemann (1955) Saint and Hunt (1955) Horsfall (1956) and de Gruchy Crawford and Morton (1958) are also probable examples Cases 2 3 and 5 of Dacie and co workers (1953) were the prototypes of Type I Most of the other cases in the literature which have been mentioned above also are probably examples of Type I

In the following section an attempt will be made to summarize the clinical and hæmatological findings of hereditary non sphero cytic hæmolytic anæmia Types I and II cannot be distinguished clinically with any certainty

Clinical Features of Hereditary Non Spherocytic Hæmolytic Anæmia

Inheritance The available evidence suggests that hereditary non spherocytic hæmolytic anæmia is inherited as a Mendelian dominant If so the penetrance of the gene clearly varies considerably (*e g* Smiley *et al* 1956) Not infrequently too both parents of an affected child appear normal (see below) Mutation seems an unlikely explanation in such cases as more than one sibling may be affected (*e g* Dacie *et al* 1953 Motulsky Crosby and Rappaport 1954 Case 4) When the cases described in the literature reviewed above and the authors personally studied cases are considered together it will be found that in only seven out of 35 families was a parent of the propositus proved by an unequivocal history and laboratory tests to be suffering from the same disease as his or her child in five other families one parent may have been very mildly affected (*e g* Cases 3 and 5 of Dacie *et al* 1953) More than one sibling was affected in 18 out of the 35 families

Both sexes are affected and there is no evidence that hereditary *non spherocytic hæmolytic anæmia is more common in any* particular racial group An association with group A was pointed out by Crosby (1950) The three anæmic members of the large family studied by Smiley and co workers (1956) were also group A but splenomegaly (without anæmia) in other members of this family did not seem to be confined to any particular ABO or Rh group This possible association with group A needs further study

Age of onset Most of the published case reports have dealt with children who have usually given a history of jaundice or

Table 8

Hæmatolo₀ical Data in Patients sufferin₀ from Hereditary Non Spherocytic Hæmolytic Anæmia

Type of hereditary non spherocytic hæmolytic anæmia	Erythrocytes (minimum counts) (mill /cu mm)		Hæmoglobin (minimum values) (g /100 ml)		MCV (cu μ)		MCHC (%)		Reticulocytes (maximum counts) (%)		Serum bilirubin (maximum values) (mg /100 ml)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
I (1?)*	1 ?–5 0	3 1	6 3–13 1	9 8	70–116	100	27–37	31 ?	3 7–?3	11 ?	0 7–? 1	? 3
II (?)†	1 5–1 9	1 7	6 2– ? ?	6 7	111–156	131	27–34	30 8	40–75	?6	1 8–2 0	1 9

The figures in parenthesis indicate the number of patients investigated

* Splenectomy had not been carried out.

† After splenectomy

small numbers Polychromasia is recognizable according to the height of the reticulocyte count and in some patients punctate basophilia may be found Target cells may be conspicuous after splenectomy (Fig 75)

Siderocytes were present in very large numbers after splenectomy in the author s two Type II cases (Fig 69) The reticulocyte count is usually substantially raised and may be extremely high (Table 8) Nucleated erythrocytes are often present in small numbers in the more anæmic patients The MCV ranges from the normal level to very high values the mean for the author s 12 Type I cases (not splenectomized) was 100 cu μ and for the two Type II cases 131 cu μ The hæmoglobin concentration varies from very low levels (2 8 g Nelson (1954)) to almost normal The mean minimum values of the author s patients was 1 6 g lower than in his series of patients with hereditary spherocytosis (Tables 5 and 8) The MCHC is normal or slightly lowered (Table 8) The serum bilirubin concentration usually lies between a normal level and 5 mg per 100 ml

The leucocyte and platelet counts are usually normal (before splenectomy)

Osmotic Fragility *Before incubation* Osmotic fragility is characteristically normal However in five out of 15 patients studied by the author there were small tails of fragile cells (Fig 72) Even so the median fragility (MCF) of these patients was not increased (*cf* hereditary spherocytosis in which if the fragility is increased at all this is reflected in an increase in median fragility)

After incubation at 37 C for 24 hours In the two Type II cases there was a substantial increase in fragility (*i e* marked lysis in 0 85% NaCl and a definite increase in MCF (Fig 72) In five other patients (Type I) there were small tails of unusually fragile cells (*i e* lysis in 0 8% NaCl) but in three of these patients and in several others the curve as a whole was shifted to the resistant side in none was there a general shift towards increased fragility

Autohæmolysis The results of studies in fifteen cases are shown in Table 9 These data include those previously published by Dacie and co workers (1953) and Selwyn and Dacie (1954) In the Type II cases autohæmolysis was markedly increased both at 24 hours and 48 hours and the addition of glucose increased rather than diminished lysis In the remaining Type I cases autohæmolysis was normal or only slightly increased and the addition of glucose reduced hæmolysis substantially but usually

less than with normal erythrocytes Abnormal autohæmolysis has also been reported by Motulsky Crosby and Rappaport (1954) and Brafield and Reef (1957) The differences between Type I and Type II cases in respect of osmotic fragility and autohæmolysis are summarized and contrasted with the findings in hereditary spherocytosis in Table 10 (see also Fig 40 p 97)

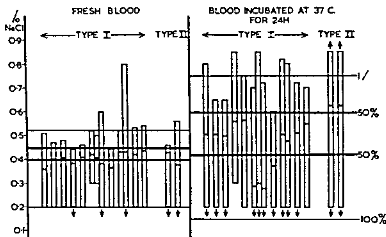


FIG 7. The results of osmotic fragility tests carried out on 15 patients suffering from hereditary non-spherocytic hæmolytic anæmia

Left hand chart results with fresh blood. *Right hand chart* results with blood incubated for 24 hours at 37 C. See also legends to Figs 40 and 5?

Mechanical Fragility This has usually been reported as normal or slightly increased Increased mechanical fragility has been observed more frequently after incubation (Dacie *et al* 1953 Motulsky Crosby and Rappaport 1954 Hennemann 1955 Smiley *et al* 1956)

Abnormal Hæmoglobins In all the cases so far studied the hæmoglobin has been reported as being of the normal type Fœtal hæmoglobin has been detected in addition in the blood of infants but not apparently in excessive amounts

White Beaven and Ellis (1959) have by now measured the fœtal hæmoglobin content in 17 cases of different types of congenital non spherocytic hæmolytic anæmia in only one was an abnormal amount (2%) detected

Table 9

Autohæmolysis in Hereditary Non Spherocytic Hæmolytic Anæmia

Case No Family	% Hæmolysis			
	24 hr (No added glucose)	24 hr (With added glucose)	48 hr (No added glucose)	48 hr (With added glucose)
	Type I Cases			
1 O F *	0 8	0 2	4 1	0 7
2 G S	<0 1	0 1	0 7	0 5
G S *	0 2	0 1	1 6	0 5
3 A Mc	0 7	0 4	3 5	2 5
4 V W	0 6	0 2	1 3	0 9
5 I L †	0 9	0 25	5 9	2 1
6 Mrs P	0 8	0 3	2 4	1 1
7 J P	0 9	0 3	2 4	0 9
8 A F *	2 4	0 9	3 0	1 9
9 C Y †	0 6	0 2	4 0	0 8
10 R F †	0 7	0 45	4 7	3 0
11 S M	1 8	1 3	5 0	4 1
12 A W	0 8	1 0	4 0	3 5
13 C F	0 8	0 7	3 0	2 0
	Type II Cases			
14 M C *†	5 3	7 2	40	46
15 J M *†	5 4	7 4	32	35
Normal Range	0 05–0 5	0–0 4	0 4–4 5	0 03–0 4

* After splenectomy

† Mean values (more than one observation available)

own circulation Kruyt and his co workers (1956) obtained ⁵¹Cr half times of 9 days and 6 days respectively in auto transfusion experiments carried out on two children

Survival studies of erythrocytes in their own environment have been carried out in five of the author s patients using ⁵¹Cr (Lewis Szur and Dacie 1959) The mean cell life spans were estimated to be 5 6 5 12 24 and 25 days respectively (see Fig 73) The two extremely short survival times were obtained in Case 3 (Type I 6 5 days) and Case 4 (Type II 5 days) of Dacie and co workers (1953) The remaining patients were Type I cases

Treatment of Hereditary Non Spherocytic Hæmolytic Anæmia

Blood Transfusion As there is good evidence that transfused normal erythrocytes survive well in the circulation of patients with hereditary non spherocytic hæmolytic anæmia transfusion is a rational and most useful method of treatment In some patients transfusion at intervals may be essential to maintain life most patients fortunately usually manage to maintain an equilibrium between erythrocyte destruction and production and achieve hæmoglobin levels of 7 5 g per 100 ml or more Such patients are best not transfused

Steroids There is no evidence that treatment with ACTH or cortisone is of any value Nelson (1954) for instance gave 150 mg of cortisone daily for 8 days to a girl 8 years of age he noted that the platelet leucocyte and reticulocyte counts increased but the total erythrocyte count and bilirubin levels were unaltered Kruyt and co workers (1956) treated a girl aged 6 years with cortisone and ACTH for 13 weeks Well marked Cushing type changes resulted but hæmolysis persisted unchanged Brafield and Reef (1957) similarly gave cortisone in 100 mg daily doses to an infant aged about 1 year but without any clear benefit

Splenectomy There is no doubt that splenectomy is much less effective in reducing hæmolysis than in hereditary spherocytosis or hereditary elliptocytosis Indeed patients often do not come to be diagnosed as suffering from hereditary non spherocytic hæmolytic anæmia until splenectomy has been proved to be a failure Some authors have concluded that the operation is probably contra indicated (Motulsky Crosby and Rappaport 1954) However there are a number of reports in the literature which suggest that limited improvement sometimes follows splenectomy As this is a most important point some of these case reports will be mentioned in detail

The child described by Lipton Grossman and Richmond (1953) was considerably improved by splenectomy she stabilized her hæmoglobin

Serology There is no evidence that the hæmolytic process is normally in any way connected with auto antibody formation In particular the antiglobulin test is negative Kaplan and Zuelzer s (1950b) patient (Case 3) in whom a warm auto agglutinin was demonstrated in a hæmolytic crisis is clearly exceptional

Erythrocyte Life-Span The survival of normal erythrocytes transfused to patients with hereditary non spherocytic hæmolytic anæmia is typically normal (Dacie *et al* 1953) (see Fig 67 p 174) The survival of patients corpuscles has been estimated in a normal

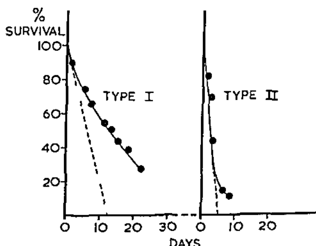


FIG 73 ^{51}Cr erythrocyte survival curves after correction for elution of chromium in two patients with hereditary non spherocytic hæmolytic anæmia The patients own cells were labelled
The Type I case = Case 9 (Table 9) mean cell life 12 days
The Type II case = Case 15 (Table 9) mean cell life 5 days
See also legend to Fig 28 (p 58)

environment by the Ashby method or in their own circulations after tagging the cells with ^{51}Cr Both methods as is to be expected, have demonstrated a marked impairment of survival

Crosby (1950) reported that the erythrocytes of his patient when transfused to a healthy recipient were eliminated in 12 days and Kaplan and Zuelzer (1950b) reported that 50 % of the erythrocytes were eliminated in 10 days and 80 % eliminated in 14 days in two patients respectively Motulsky Crosby and Rappaport (1954) also reported observations on two patients Normal erythrocytes survived normally in both The cells of one patient had a mean life span of 13 days in a healthy recipient and those of the other patient were estimated using the ^{51}Cr method to have a mean life span of 17 days in the patient s

author s patients (Type I) have been studied in this way The pattern was the same in all of them there was no evidence of any excessive accumulation of chromium in the spleen and the spleen liver ratio remained obstinately at zero (Fig 74 *cf* Figs 32 and 62 pp 66 and 162) Splenectomy has not been carried out in the above mentioned patients and there has been no opportunity of putting to the test the prediction that the operation would be of little or no benefit to them Surface counting should certainly be carried out if technically feasible whenever splenectomy is contemplated Recently Motulsky Casserd Giblett Broun and

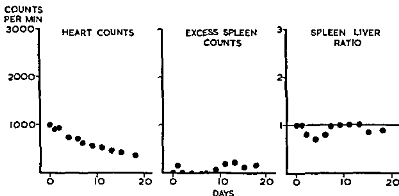


FIG 74 Results of in vivo surface counting after the labelling with Cr of the erythrocytes of a patient with hereditary non spherocytic hæmolytic anæmia (Type I) (Case 9 Table 9) The figure shows the heart counts the excess of spleen counts over the heart counts and the ratio of the excess spleen counts to the excess liver counts (spleen liver ratio) There is no significant uptake of chromium by the spleen

Finch (1958) have also reported that they failed in two patients with hereditary non spherocytic hæmolytic anæmia to detect any splenic uptake of ^{51}Cr by in vivo counting

Splenectomy has a similar effect on the blood picture as in other hæmolytic disorders *i e* there is a tendency to leucocytosis and often marked persistent thrombocytosis siderocytes and Howell Jolly bodies and occasional normoblasts are found in the circulating blood and there is sometimes marked target cell formation Schistocytes and markedly distorted cells may be found in films in small numbers

level at 8–9 g per 100 ml after operation and needed no more transfusions Before operation she had been transfused at 2–4 week intervals Her spleen was large (for a child aged 5½ years) as it weighed 330 g

Nelson s (1954) patient was also a little girl (aged 7 years) Before splenectomy the hæmoglobin ranged from 2 8–7 4 g per 100 ml over a 4 month period during the first 4 years after splenectomy it ranged between 8 4 and 10 1 g per 100 ml

The patient described as Case 3 by Motulsky Crosby and Rappaport (1954) showed only a slight improvement in hæmoglobin level after splenectomy *i e* a rise from 12 4 g to 13 5 g per 100 ml 2½ months after operation The fæcal urobilinogen excretion however diminished significantly—from 1 230 mg to 720 mg per 24 hours (means of 23 and 33 observations respectively)

Two of the patients of Smiley and co workers (1956) underwent splenectomy Both were considered to have benefited and their hæmoglobins stabilized at 10–11 g afterwards In one patient the urobilinogen excretion in the fæces was diminished after splenectomy (two 4 day collections) In one of the children it was also demonstrated that whereas before splenectomy the survival of normal erythrocytes was moderately shortened (half life 32 days) after splenectomy this became normal An extracorpuscular mechanism of hæmolysis in addition to an intrinsic erythrocyte defect was postulated

Krivit and co workers (1956) have also reported that the survival of normal erythrocytes transfused to two patients (children aged 6 and 3 years respectively) was slightly impaired One of these children was submitted to splenectomy but improvement was only temporary

Splenectomy was carried out on the infants reported by Brafield and Reef (1957) when they were 9 months and 2 months old respectively Whereas transfusions were essential before splenectomy after operation both children managed to achieve equilibrium with hæmoglobin levels of about 10–12 5 g per 100 ml

The above mentioned reports are slightly encouraging They certainly suggest that the spleen is one of the sites where hæmolysis takes place and that a certain degree of improvement may often be expected after its removal In most cases however improvement seems likely to be slight and not necessarily well sustained Nevertheless in a child who requires repeated transfusions it would seem reasonable to consider splenectomy seriously However in patients capable of attaining equilibrium at hæmoglobin levels of 8 g per 100 ml or more the operation hardly seems justifiable in view of the risks of subsequent infection (Horsfall 1956) and venous thrombosis and pulmonary embolism (Dacie *et al* 1953 Case 3 see p 174)

Selection of Patients for Splenectomy The recent introduction of *in vivo* surface counting after tagging a patient s erythrocytes with ^{51}Cr (see p 65) provides a possible means of assessing the importance of the spleen in hæmolysis Three of the



Fig. 7a. Photomicrograph of a blood film of a patient suffering from hereditary non-spherocytic haemolytic anaemia (Type I) (Case 3 of Dacie *et al.* 1953) (Case 1, Table 9). Splenectomy had been carried out 4 years previously. Macrocytosis and target cells are conspicuous. (× 700.)

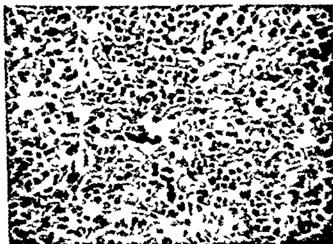


Fig. 7b. Photomicrograph of a section of the spleen of a patient suffering from hereditary non-spherocytic haemolytic anaemia (Type I) (Case 3 of Dacie *et al.* 1953). The splenic pulp is moderately congested with blood but not to the degree characteristic of hereditary spherocytosis (cf. Fig. 4.)

To face p. 188

Histology of the Spleen

There is general agreement that the spleen in hereditary non spherocytic hæmolytic anæmia differs considerably in histology from that of hereditary spherocytosis there is as a rule much less congestion of the pulp more reticulum cell hyperplasia and more extramedullary erythropoiesis There are however individual variations Whether these variations are reflections of distinct disorders is as yet uncertain

Lipton Grossman and Richmond (1953) studied a spleen (weight 330 g) removed from a child aged 5½ years the pulp was increased in extent and it contained considerable numbers of erythrocytes It is interesting to note in view of the pulp congestion that this child (and a sister) responded relatively well to splenectomy

Nelson (1954) reported that the spleen pulp of his patient was cellular but not congested with blood The littoral cells of the sinuses were conspicuous and there was an increase in the number of reticulum cells in the pulp but no obvious evidence of erythrophagocytosis

Motulsky Crosby and Rappaport (1954) described the morphology of the spleen in three cases The findings were similar in each increased cellularity of the pulp cords due to the presence of lymphocytes monocytes and macrophages but not to erythrocytes distinct sinuses with the littoral cells not particularly conspicuous increase of reticulin fibres and excess hæmosiderin

Smiley and co workers (1956) studied two spleens (weight 240 g and 300 g) removed from children aged 4 and 2½ years respectively The general pattern was normal the pulp was not congested erythrophago cytosis and extramedullary hæmopoiesis could be seen and there was an increase in reticulin fibres

A photomicrograph of the spleen of one of the author s patients (Dacie *et al* 1953 Case 5 see p 175) is illustrated in Fig 76

Classification and Pathogenesis of Hereditary Non Spherocytic Hæmolytic Anæmia

Classification As already indicated hereditary non sphero cytic hæmolytic anæmia can be separated on hæmatological grounds into at least two distinct disease entities Clinically however no such separation seems possible The majority of the patients belong to a fairly homogenous group termed Type I by Selwyn and Dacie (1954) These patients have round to elliptic erythrocytes with minor degrees of anisocytosis and poikilocytosis The osmotic fragility of fresh blood is normal but there is a tendency to abnormally increased resistance after incubation autohæmolysis rates are within the normal range or only slightly increased The Type II cases have rounded macrocytes and there is little poikilocytosis the osmotic fragility of fresh blood is normal but this increases markedly on incubation and the rate of autohæmolysis is strikingly increased (see below and Table 10)

Another possible difference between the two groups lies in the

Table 10

Morphological and Other Differences between the Erythrocytes in Hereditary Non-Spherocytic Hæmolytic Anæmia Types I and II and Hereditary Spherocytosis

Disease	Erythrocytes	Osmotic fragility		Autohæmolysis (48 hr at 37° C)	
		Before incubation	After incubation (24 hr at 37° C)	Without added glucose	With added glucose
Type I non-spherocytic	Round, oval or slightly elliptical macrocytes	Normal	Increased but not more than normal. Fragility of some cells diminished	Normal or slightly increased	Diminished but usually by less than normal amount
Type II non-spherocytic	Round macrocytes	Normal	Greatly increased	Greatly increased (×10–20 normal)	Not diminished by glucose
Hereditary spherocytosis	Round microspherocytes	Increased	Greatly increased	Increased (×5–10 normal)	Diminished by normal amount

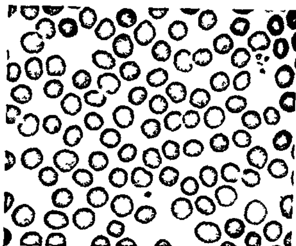


Fig 77 Photomicrograph of a blood film of a patient suffering from a congenital non spherocytic hæmolytic anæmia (unclassified type) (Case 6 of Dacie *et al* 1953) The erythrocytes are conspicuously rounded and slightly microcytic × 700

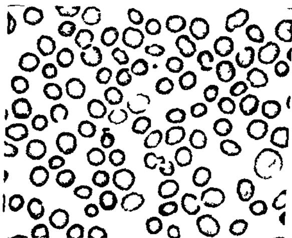


Fig 78 Photomicrograph of a blood film of a patient suffering from a (?) congenital hæmolytic anæmia (unclassified type) Round or oval microcytes and crenated cells can be seen There is also a tendency to spherocytosis × 700

Table 10

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Hereditary spherocytosis	Round microspherocytes	Increased	Greatly increased	Increased (×5–10 normal)	Diminished by normal amount

reticulocyte counts i e in the bone marrow responses In the two Type II patients studied by the author the reticulocyte counts have been very high indeed (mean 56%) as was that of Hennemann s (1955) (? Type II) patient whose erythrocytes were also reported to have undergone rapid autohæmolysis The reticulocyte counts of the four patients of de Gruchy Crawford and Morton (1958) (thought to be Type II) ranged between 30 % and 60 % Counts as high as these do not seem to have been reported in Type I patients (It seems possible that the regenerative capacity of the bone marrow is less good in Type I patients) It does not seem likely that the differences in reticulocyte counts can be explained solely on the severity of hæmolysis in the Type II patients If the stimulus to erythropoiesis is quantitatively related to the hæmoglobin concentration in the peripheral blood then the reticulocyte counts of Cases 3 and 4 of Dacie and his colleagues (1953) should have been about the same Actually Case 4 with a mean cell life span of 5 days regularly had counts exceeding 50% while Case 3 with a mean cell life span almost as short (6 5 days) had counts of only 5–9%

The patients described as having increased punctate basophilia may belong to a separate group (*e g* the patients of Haden (1947 2nd family) Feinberg and Watson (1951) and Kruvit *et al* (1956 Case 1) *Those in whom erythrocyte morphology* is grossly abnormal (*e g*, Case 10 of Dacie *et al* (1953)) seem also likely to be representative of rare distinct entities (see p 193)

Pathogenesis Very little is known of the nature of the erythrocyte defects in hæmolytic anæmias of the type now being considered or how the defects shorten the life span of the corpuscles *in vivo* It is certain however that the mechanism of hæmolysis differs from that of hereditary spherocytosis in particular, (a rapid rate of erythrocyte destruction is not dependent upon the presence of a spleen as is shown by the fact that splenectomy is of little therapeutic value)

Some light was thrown on the problem by the studies of Selwyn *and Dacie (1954) As already mentioned it was found* possible to separate the patients into two groups by means of studies *in vitro* On incubation the cell volume and cation changes of *the Group I cases were similar to those of normal corpuscles* as was the increase in osmotic fragility on incubation and the rate of autohæmolysis However a definite abnormality was demonstrable for when glucose was added to the blood the rate of autohæmolysis was diminished by less than the normal amount Further data on Group I patients are given in Table 9

The erythrocytes of the Group II patients behaved quite differently from those of Group I During incubation the potassium losses were much greater than normal and the cation and cell volume changes were unaffected by the addition of glucose Autohæmolysis was markedly increased in both the patients studied and this was similarly unaffected by glucose Further experiments indicated that the erythrocytes of these patients were unable to utilize glucose at the normal rate—the observed utilization was only 25% and 30% respectively of the calculated amounts These observations demonstrated that the erythrocytes of the two groups of non spherocytic cases and the erythrocytes of hereditary spherocytosis all behave differently on incubation *in vitro* The essential nature of the corpuscular defects could not however be determined

Metabolic Studies Several patients have more recently been studied by techniques similar to those which have demonstrated a metabolic defect in hereditary spherocytosis (see p 183)

Motulsky Gabrio Burkhardt and Finch (1955) mentioned that although the ^{32}P uptake and glycolytic rates of mature erythrocytes were normal in non-spherocytic hæmolytic anæmia disturbed intra cellular distribution of phosphorylated intermediates was detected in several cases in particular they mentioned that increases in 2 3 diphosphoglycerate (2 3 DPG) were found Prankerd (1957) gave brief biochemical data on four patients The results in one patient were normal the others showed gross defects *i e* low adenosine triphosphate (ATP) and 2 3 DPG deficient glucose consumption and decreased ^{32}P-orthophosphate exchange

de Gruchy Crawford and Morton (1958) have reported briefly on the results obtained in four (Type II) patients ATP was decreased but the total organic phosphate was markedly increased probably due to an increase in 2 3 DPG Similar studies on the erythrocytes in hereditary spherocytosis gave a markedly different pattern of results ATP was found also to reduce the rate of in vitro autohæmolysis although glucose failed to do this

Further studies correlated with the clinical and hæmatological findings are clearly needed but there seems no reason to question the view that the essential defect in the erythrocytes of hereditary non spherocytic hæmolytic anæmia is a biochemical one involving the glycolytic mechanism

Differential Diagnosis

The hereditary (congenital) non spherocytic hæmolytic anæmias are not as a rule difficult to diagnose The following disorders have to be considered hæmolytic disease of the newborn hereditary spherocytosis with normal osmotic fragility hereditary elliptocytosis Mediterranean anæmia familial non hæmolytic jaundice (constitutional hyperbilirubinæmia)

Hæmolytic Disease of the Newborn In cases of apparent hæmolytic disease of the newborn with negative serological findings hereditary non spherocytic hæmolytic anæmia should be thought of The blood pictures may be identical The future progress of the child will of course decide the issue

Hereditary Spherocytosis with Normal Osmotic Fragility As already mentioned (p 108) the author is coming round to the view that most cases of hereditary spherocytosis with normal osmotic fragility are probably not examples of hereditary spherocytosis at all Autohæmolysis and osmotic fragility estimation after incubation should allow clear cut differentiation between non spherocytic hæmolytic anæmia and the mildest cases of true hereditary spherocytosis Comparison of the blood pictures should also help rounded normocytes and microcytes with few ovalocytes and poikilocytes being characteristic of hereditary spherocytosis

Hereditary Elliptocytosis The distinction between hereditary elliptocytosis with overt hæmolysis and non spherocytic hæmolytic anæmia can usually be made simply on morphological grounds In the former the majority of erythrocytes are conspicuously elliptic or oval in the latter only a minority of the cells are elliptic Occasionally however the distinction is difficult (*cf* Figs 68 and 70 with Figs 55 and 57) and it is possible that certain apparent Type I non spherocytic cases showing minor degrees of elliptocytosis are in fact variants of hereditary elliptocytosis Family studies may help in the differentiation

Mediterranean Anæmia (Thalassæmia) There should be little confusion with Mediterranean anæmia In thalassæmia major the blood picture is quite distinct and large amounts of fœtal hæmoglobin are present In thalassæmia minor hypochromia of the erythrocytes is more marked than in the hereditary non spherocytic hæmolytic anæmias and the degree of anisopoikilocytosis is generally greater In addition there is usually less evidence of hæmolysis and the reticulocyte counts are lower in thalassæmia minor Family studies are also of great help

Familial Non Hæmolytic Jaundice According to Motulsky Crosby and Rappaport (1954) familial non hæmolytic jaundice is a syndrome brought about by several mechanisms but they believe that some patients diagnosed as constitutional hyperbilirubinæmia may have in fact been suffering from non spherocytic hæmolytic disease Normally however confusion should not arise but it is obvious that clear evidence of hæmolysis should be sought for in a patient with a hereditary jaundice before

assuming that the jaundice is due to excessive hæmolysis and *vice versa* On the other hand there are patients really suffering from excessive hæmolysis in whom jaundice is unusually conspicuous (*e g* Case 5 of Dacie *et al* 1953) Such patients possibly have (? constitutional) hepatic dysfunction in addition to defective erythrocytes (see also p 89)

MISCELLANEOUS TYPES OF ATYPICAL CONGENITAL HÆMOLYTIC ANÆMIA

In the following section certain rare types of congenital hæmolytic anæmia which do not fit easily into any of the categories so far mentioned will be briefly discussed They are tentatively grouped as follows

✓1 *Cases Resembling Hereditary Spherocytosis but with Normal Osmotic Fragility and a Normal or Almost Normal Rate of Autohæmolysis*

Cases 6–9 of Dacie and his co workers (1953) are believed to belong to this group Case 6 has been re investigated several times between 1952 and 1958 On each occasion the osmotic fragility before and after incubation has been clearly normal and the rate of autohæmolysis normal or only just above normal (hæmolysis at 48 hours 2 2–5 6 / 0 1–1 0 / with added glucose) His clinical condition is unchanged he is not anæmic although slightly jaundiced and the reticulocyte count has remained at about 6 / His red cells are normocytic (MCV 90 cu. μ MCD 7 0 μ) and round in contour they look slightly spherocytic (Fig 77)

✓ 2 *Cases Resembling Hereditary Spherocytosis Osmotic Fragility Increased but Blood Picture Atypical*

A curious type of erythrocyte fragmentation (pincered cells) was observed by Dacie and co-workers (1953) in a patient (Case 7) with an hereditary hæmolytic anæmia believed to belong to the preceding category A further patient showing the same phenomenon has recently been studied In this instance however there was in addition unequivocal microspherocytosis and increased osmotic fragility both before and after incubation (Fig 78)

✓3 *Cases without Spherocytosis but with Bizarre Blood Pictures*

In Fig 79 is illustrated the blood film of a remarkable case The owner of the film a young man of 19 years was described by Dacie and co workers (1953) as having a macrocytic type of congenital hæmolytic anæmia He then gave a 4 years history of continuous jaundice In addition he complained of small indolent ulcers on his shins and above his ankles His erythrocytes were unusually macrocytic and varied considerably in size and shape Their osmotic fragility was normal or slightly diminished No definite evidence of a familial incidence could be established

Splenectomy was carried out but without improving his anæmia His jaundice however was slightly lessened After operation his erythrocytes became even more macrocytic (MCD 9 3 μ) An unusual

Hæmolytic Disease of the Newborn In cases of apparent hæmolytic disease of the newborn with negative serological findings hereditary non spherocytic hæmolytic anæmia should be thought of The blood pictures may be identical The future progress of the child will of course decide the issue

"*Hereditary Spherocytosis with Normal Osmotic Fragility* As already mentioned (p 108) the author is coming round to the view that most cases of hereditary spherocytosis with normal osmotic fragility are probably not examples of hereditary spherocytosis at all Autohæmolysis and osmotic fragility estimation after incubation should allow clear cut differentiation between non spherocytic hæmolytic anæmia and the mildest cases of true hereditary spherocytosis Comparison of the blood pictures should also help rounded normocytes and microcytes with few ovalocytes and poikilocytes being characteristic of hereditary spherocytosis

Hereditary Elliptocytosis The distinction between hereditary elliptocytosis with overt hæmolysis and non spherocytic hæmolytic anæmia can usually be made simply on morphological grounds In the former the majority of erythrocytes are conspicuously elliptic or oval in the latter only a minority of the cells are elliptic Occasionally however the distinction is difficult (*cf* Figs 68 and 70 with Figs 55 and 57) and it is possible that certain apparent Type I non spherocytic cases showing minor degrees of elliptocytosis are in fact variants of hereditary elliptocytosis Family studies may help in the differentiation

Mediterranean Anæmia (Thalassæmia) There should be little confusion with Mediterranean anæmia In thalassæmia major the blood picture is quite distinct and large amounts of fœtal hæmoglobin are present In thalassæmia minor hypochromia of the erythrocytes is more marked than in the hereditary non spherocytic hæmolytic anæmias and the degree of anisopoikilocytosis is generally greater In addition there is usually less evidence of hæmolysis and the reticulocyte counts are lower in thalassæmia minor Family studies are also of great help

Familial Non Hæmolytic Jaundice According to Motulsky Crosby and Rappaport (1954) familial non hæmolytic jaundice is a syndrome brought about by several mechanisms but they believe that some patients diagnosed as constitutional hyperbilirubinæmia may have in fact been suffering from non spherocytic hæmolytic disease Normally however confusion should not arise but it is obvious that clear evidence of hæmolysis should be sought for in a patient with a hereditary jaundice before



Fig 70 Photomicrograph of a blood film of a patient suffering from a macrocytic type of congenital hæmolytic anæmia (Case 10 of Dacie *et al* 1953) Eight years after splenectomy The erythrocytes vary greatly in size and shape Cell fragments are present × 00

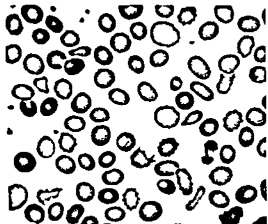


Fig 80 Photomicrograph of a blood film of a child suffering from a congenital hæmolytic anæmia (unclassified type) The erythrocytes vary markedly in size and a few poikilocytes are present also × 00

feature in the bone marrow was the presence of quite large numbers of plurinucleated erythroblasts The plurinucleated erythroblasts and the unusual degree of macrocytosis and poikilocytosis in the peripheral blood suggested that the diminished life span of the erythrocytes was secondary to some unusual defect in erythropoiesis

The type of macrocytic anæmia described above is believed to be rare It is possible that the patients described by Fanconi (1940) and Vecchio and Tropeano (1947) were suffering from a somewhat similar disorder Splenectomy was ineffective in Fanconi s patient

In Fig 80 is illustrated the blood film of another unusual case of congenital hæmolytic anæmia The patient was a female infant aged 9 months The film shows a moderate degree of anisopoikilocytosis with occasional macrocytes microcytes and cell fragments The osmotic fragility was normal both before and after incubation No abnormal hæmoglobins were present The blood of both parents was normal

Congenital Hæmolytic Anæmia Associated with Porphyria (Erythropoietic Porphyria)

There are a small number of recorded instances of hæmolytic anæmia associated with congenital porphyria (e g de Marval and Pons 1934 Aldrich Hawkinson Grinstein and Watson 1951 Gray and Neuberger 1952 Schmid Schwartz and Watson 1954 Schmid Schwartz and Sundberg 1955 Rosenthal Lipton and Asrow 1955 Varadi 1958 Stich 1958)

Splenectomy was carried out in de Marval and Pons s patient Following operation hæmolysis was greatly reduced and the photo sensitivity of the skin became less marked The patient of Aldrich and his associates was a little girl aged 4 years Her spleen was also removed Before the operation she was severely anæmic her erythrocytes were slightly macrocytic some were said to be small and spherocytic and curious granulation was noted in the circulating erythrocytes and in the normoblasts in the marrow Following splenectomy her anæmia disappeared and so did the photosensitivity The authors attributed this to a reduction in the synthesis of porphyrins associated with the diminution in erythropoiesis following alleviation of the hæmolytic process Splenectomy was also carried out on Gray and Neuberger s patient (an adult) in this instance however neither the blood picture nor the photosensitivity was favourably affected

Schmid Schwartz and Watson (1954) in the course of discussing the porphyrin content of the bone marrow and liver in various forms of porphyria referred to two patients with erythropoietic porphyria who underwent splenectomy In both the excretion of porphyrins diminished and photosensitivity was improved Schmid Schwartz and Sundberg (1955) reported on the bone marrow findings in five cases of erythropoietic porphyria and illustrated nuclear fluorescence and nuclear abnormalities in normoblasts containing porphyrins They also reviewed the literature Thirty four cases of erythropoietic porphyria were accepted as genuine of these sixteen were reported to have hæmolytic anæmia and most of the remainder were known to be anæmic

Varadi's (1958) patient was a female infant aged 4½ months whose presenting symptoms and signs were pallor the passage of red urine and splenomegaly Staining defects in the nuclei of normoblasts were noted and in about 10% of the cells fine needle-like structures (? crystals of porphyrins) were visible Some of the polychromatic cells in the peripheral blood similarly showed fine needle like structures when stained by Romanowsky dyes Varadi also illustrates (his Fig 12) the marked fluorescence in ultraviolet light of the nuclei of many of the infant's normoblasts Splenectomy was followed by a marked diminution in the rate of hæmolysis The symptom of photosensitivity was also alleviated Varadi suggested in discussing the generally beneficial effect of splenectomy that the hæmolytic process is originally confined to a selective elimination of porphyrin laden cells by the spleen and that hypersplenism develops later

Stich (1958) also described a severely affected infant who benefited markedly from splenectomy

Congenital Hæmolytic Anæmia Associated with Heinz Body Formation

Congenital hæmolytic anæmia associated with marked Heinz body formation occurs in two forms as a transient (although sometimes fatal) disorder of the neonatal period predominantly affecting premature infants and as a very rare disorder persisting into later childhood Some at least of the cases of the first group appear to have been due to the administration of too large doses of vitamin K For this reason they will be considered in Chapter 15 The latter group of cases are dealt with below They are almost certainly caused by a congenital defect of the erythrocytes

Congenital Heinz Body Anæmia

Very few patients have as yet been studied none adequately before splenectomy The first available report is that of Cathie (195)

The affected child was born 5 weeks prematurely and had always been pale and jaundiced He was first investigated in hospital when 16 months of age His hæmoglobin was 7 g per 100 ml and there were 37% of reticulocytes Erythrocyte osmotic fragility was increased Hæmolytic anæmia was diagnosed and splenectomy performed in June 1948 after a preliminary blood transfusion Four months later the child was readmitted with a recurrence of anæmia the reticulocyte count being 75%

In January 1950 many of his corpuscles were found to contain large Heinz bodies and Romanowsky stained blood films showed almost exactly the same abnormalities as did films of the author's patient (Dacie 1954 Fig 92) who was suffering from acetylphenylhydrazine poisoning (after splenectomy) Many of the corpuscles were crenated and shrunken and some of these contained recognizable palely basophilic structures of the size of Heinz bodies Punctate basophilia and diffuse polychromasia were striking features and Pappenheimer bodies were numerous No cause for the development of the Heinz bodies



FIG. 81. Photomicrograph of a blood film of a child suffering from congenital Heinz body anaemia. There is marked basophilic stippling and some of the smaller crenated cells contain visible Heinz bodies. × 700

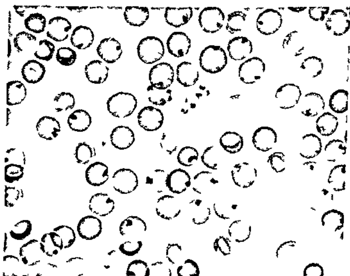


FIG. 82. Photomicrograph of the erythrocytes of a patient suffering from a (?) congenital hæmolytic anæmia (unclassified type). Splenectomy had been carried out 18 years previously. Many of the cells contain large Heinz bodies. Wet preparation stained with methyl violet. × 700

Three months after splenectomy 48 / of A F s erythrocytes contained Heinz bodies Romanowsky stained films showed numerous Pappen heimer bodies and cells with diffuse punctate basophilia were infrequent although present Heinz bodies were just visible in many of the cells as clear areas surrounded with darker staining rims There was no undue tendency to cell contraction or crenation The erythrocytes of the other (non splenectomized) affected members of the family did not contain Heinz bodies

The other patient kindly referred to me by Dr F Hemsted is a man aged 43 who had undergone splenectomy 18 years previously He gave a history of periodic attacks of jaundice and splenomegaly since childhood After splenectomy 37–40 of his erythrocytes were found to contain large Heinz bodies (Fig 82) He was slightly anæmic (hæmoglobin 13 1–13 4 g per 100 ml) and there were 3–5 of reticulo cytes Osmotic fragility was normal Many of the erythrocytes contained Pappenheimer bodies but there was no punctate basophilia A small proportion of the Heinz bodies were just visible but there were no pathologically contracted cells present

The two cases of Heinz body anæmia just referred to do not easily fit into any known category They seem however to provide a link between the severe congenital Heinz body anæmias of early childhood and some cases of Type I hereditary non spherocytic hæmolytic anæmia

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could be found The child was kept under strict observation but the Heinz bodies did not disappear and it seemed impossible that he was taking or being given any noxious chemical or drug

In Fig 81 is illustrated the photomicrograph of a blood film of another child suffering from probably the same disorder as that of Cathie s patient After splenectomy the blood pictures were identical Approximately 44% of the erythrocytes contained large Heinz bodies A Romanowsky stained film made before splenectomy was available for comparison Marked polychromasia was present and occasional cells showed punctate basophilia A few small contracted cells with moderately smooth outlines were present Heinz bodies were not visible and it is not known whether they were present before the spleen was removed After splenectomy nearly every cell showed some degree of punctate basophilia and small contracted crenated cells in which the outlines of Heinz bodies could be easily seen were conspicuous

A similar case was reported by Allison (1957) Enzyme studies on this child s erythrocytes revealed high values for cholinesterase and glyoxalase consistent with the reticulocytosis present but the catalase content was less than one third of control specimens Allison concluded that the low catalase activity was a manifestation of defective erythrocyte *metabolism*

Three more patients probably suffering from the same disorder have been studied in America Schmid Williams and Clemens (1958) gave an account of a 32 year old man and his son both of whom had undergone splenectomy The father had a reticulocyte count of 40–70% and the son 20–40% The mean cell life spans were 8 and 15 days respectively No abnormal hæmoglobins were demonstrable and the erythrocyte catalase was apparently normal The erythrocytes were described as containing one (occasionally two) Heinz like bodies Both patients excreted dark brown urine but the pigment could not be identified Tests for bilirubin biliverdin porphobilinogen porphyrins pentdyopent hæmochromogen indican melanin and homogentisic acid gave negative results

The patient described by Lange and Akeroyd (1958) was a 14 year old female known to be anæmic since the age of 2½ years She also had petit mal Splenectomy was carried out when she was 4½ years old When re examined 10 years later 14% of the erythrocytes contained inclusion bodies up to 2 μ in diameter The urine of this patient also contained an unusual dark pigment which Lange and Akeroyd suggested may belong to the bilifuscin and mesobilifuscin group It should be added that in Lange and Akeroyd s opinion the inclusion bodies were not Heinz bodies but represented a new form of inclusion They also reported that preliminary studies indicated that there was a possible defect in the glutathione content of the patient s erythrocytes

Large numbers of Heinz bodies have been observed by the author in two further cases of congenital non spherocytic hæmolytic anæmia (after splenectomy)

One patient A F (Case 8 Table 9) was a girl aged 11 a member of a family kindly referred to the author by Dr Louis Steingold The child s mother (Case 6 Table 9) and two sisters were also affected Only A F had undergone splenectomy and the family appeared to be suffering from typical Type I non spherocytic hæmolytic anæmia

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In 1936 Whipple and Bradford introduced the descriptive terms Mediterranean disease or thalassæmia Mediterranean anæmia (not disease) and thalassæmia are both now widely used particularly the latter because of the ease with which it may be qualified with the words major or minor (see below)

Cooley Witwer and Lee (1927) at first considered that they were dealing with a familial anæmia of hæmolytic type Later they considered that the anæmia was primarily due to a metabolic disturbance and likened the defective erythropoiesis to attempts to make bricks without straw (Cooley and Lee 1932) As already mentioned it is now realized that both mechanisms are operative and that the hæmolysis is secondary to and probably less important than defective hæmopoiesis Nevertheless Italian authors in particular have referred to patients in whom hæmolysis has seemed to be a dominant feature as suffering from itteri emolitici con aumento della resistenza globulare In Italy this type of anæmia has come to be known as hæmolytic anæmia of the Rietti Greppi Micheli type (Rietti 1946 Marmont and Bianchi 1948 Chini and Valeri 1949 de Muro and Leonardi 1950)

Inheritance It is now generally recognized that Mediterranean anæmia exists in two main grades of severity (Valentine and Neel 1944) thalassæmia major (Cooley s anæmia) a serious disorder which is usually fatal in childhood and thalassæmia minor (target cell anæmia or target oval cell syndrome (Dameshek 1940 1943) a less serious and not fatal disorder More recently Astaldi Tolentino and Sacchetti (1951) referred to the mildest form of the disease as thalassæmia minima and types intermediate between the major and minor disorders have been described (Sturgeon Itano and Bergren 1955) It is also now realized that the presence in the same subject of a gene for thalassæmia as well as a gene for an abnormal hæmoglobin can give rise to a whole series of interesting clinical syndromes These are dealt with in Chapter 6

Caminopetros (1936 1938) was the first to report that both parents of a child suffering from severe Mediterranean anæmia although they might appear healthy had erythrocytes which were abnormally resistant to hæmolysis by hypotonic saline He correctly concluded that this was a sign of a latent form of the disease This important observation was confirmed by Panoff (1936) and Angelini (1937) Angelini for instance reported that almost all the available relatives of the six patients he studied were carriers of the trait

CHAPTER 5

THE CONGENITAL HÆMOLYTIC ANÆMIAS

4 MEDITERRANEAN ANÆMIA (THALASSÆMIA) AND ALLIED DISORDERS

In this chapter will be described certain congenital anæmias due primarily to defective hæmoglobin synthesis This leads to the formation of erythrocytes very low in hæmoglobin content and usually very variable in size and shape The life span of the most defective of these corpuscles is considerably reduced For this reason in Mediterranean anæmia the most frequently encountered anæmia of this group excessive hæmolysis may be *correctly regarded as playing a part in the causation of the* patient s anæmia

MEDITERRANEAN ANÆMIA

Synonyms *Cooley s anæmia (Kato and Downey 1933)* erythroblastic anæmia (Cooley and Lee 1932) Mediterranean disease—thalassæmia (Whipple and Bradford 1936) target cell anæmia (Dameshek 1940) familial microcytic anæmia (Strauss Daland and Fox 1941) Rietti Greppi Micheli anæmia (see *Marmont and Bianchi 1948) Mediterranean hæmopathic syndrome* (Chini and Valeri 1949) hereditary leptocytosis (Committee for Clarification of Nomenclature etc 1950) Hemopathic Mediter ranean syndrome (de Muro and Leonardi 1950)

History The first descriptions of Mediterranean anæmia as a distinct entity were those of Cooley and Lee *(1925) and Cooley* Witwer and Lee (1927) who described a number of children suffering from splenomegaly with anemia and peculiar bone changes Later the eponym Cooley s anæmia was widely used in descriptions of the disease It is now realized that both serious and benign forms of the disease are *comparatively* commonly found chiefly in people of Mediterranean *origin and in* the Far East On the Continent the disorder has been extensively studied particularly in Italy and it has now a large literature (see Lehndorff 1936 Chini and Valeri 1949 Astaldi Tolentino and Sacchetti 1951)

distinct from the two other known loci concerned with normal and abnormal hæmoglobin formation Allison (1955) suggested Th^T as a possible designation for the abnormal gene Th^N being the normal allele Thus the genotype of a heterozygote would be written Th^N/Th^T and a homozygote Th^T/Th^T Neel (1958) accepted this nomenclature but remarked that the gene symbol Th^A rather than Th^N seemed to be preferred by most workers in the field

The abnormal gene for thalassæmia exists at high frequency in certain Mediterranean populations Neel and Valentine (1945) who

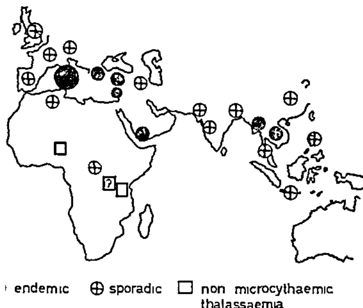


FIG 83 Map showing the distribution of Mediterranean anæmia in the Old World Redrawn from Lehmann (1959b) (For a note on non microcythæmic thalassæmia see p 268)

studied in America a population originating from South Italy and Sicily found that approximately 4% had thalassæmia minor In certain regions in Italy particularly in the Po delta Calabria Puglia and in Sicily the incidence reaches almost 10% (Silvestroni and Bianco 1959) while Banton (1951) found evidence of thalassæmia minor in approximately 20% of more than 500 Turkish and Greek Cypriot children in a small mountain village the incidence even reached 50% With such high incidences it is not clear how the gene persists Presumably a state of balanced polymorphism is brought about by environmental or other factors with the thalassæmia heterozygotes being at an advantage It is possible that two such factors are an

In America Atkinson (1939) and Wintrobe and his colleagues (1940) were similarly the first to recognize that Cooley's anæmia (as seen in the United States) existed in a less severe inheritable form

Atkinson studied two young adults aged 20 and 17 Although the blood counts of the parents were considered to be normal a marked increase in erythrocyte osmotic resistance was demonstrated Wintrobe and his colleagues reported in detail the findings in 14 patients belonging to three families of Italian parentage They found and described nearly all the signs which are now recognized to be characteristic of thalassæmia minor In a postcript they mentioned that they had been able to demonstrate similar abnormalities in the blood of both parents of a child with typical Cooley's anæmia These studies were confirmed and elaborated by Smith (1942) and Dameshek (1943) in America by Rohr (1943) in Switzerland and by Italian workers (see Chini and Valeri 1949) and it is now realized that studies on the blood of parents of children affected with Cooley's anæmia will almost invariably reveal minor but definite hæmatological abnormalities on both sides of the family Rietti (1946) reviewed earlier Italian publications on the Rietti Greppi Micheli anæmia—his own cases were first reported in 1925—and equates them with those described by Wintrobe and his colleagues There seems little reason now to doubt that cases of the so called Rietti Greppi Micheli type are in fact forms of thalassæmia minor

It is now generally agreed that the severe major form of the disease represents the homozygous state of a partially dominant autosomal gene and that the minor and minima forms represent the heterozygous condition (Valentine and Neel 1944 Smith 1948 Chini and Valeri 1949 Astaldi Tolentino and Sacchetti 1951a Banton 1951 Ludwin Limentani and Dameshek 1952 Bianco *et al* 1952 Zuelzer Neel and Robinson 1956 Neel 1956–57) Many family studies have been carried out Silvestroni Bianco and Vallisneri (1949) for instance studied 110 sibships Microcythæmia (thalassæmia minor or minima) was demonstrable in nearly all of the 220 parents in only four were the signs doubtful or negative

Zuelzer, Neel and Robinson (1956) also mentioned that rarely one parent of a child with obvious Cooley's anæmia might show no signs of thalassæmia minor and postulated a failure of expression of the gene They added that in three such families near relatives of the normal parent had unequivocal thalassæmia minor (Similar occurrences have been described in hereditary spherocytosis see p 85)

The genetics of thalassæmia have been recently discussed by Neel (1956–57) The abnormal gene for thalassæmia is found at a locus

1954 Pouya 1959) in oriental Jews (Schieber 1945 Dreyfuss 1955 Matoth Shamir and Freundlich 1955) in negroes (Schwartz and Mason 1949 Banks and Scott 1953 Schwartz and Hartz 1955 Norris Hanson and Loeffler 1956) and in Thais (Minnich *et al* 1954 Na Nakorn 1959)

CLINICAL AND HÆMATOLOGICAL FEATURES OF MEDITERRANEAN ANÆMIA

Thalassæmia Major (Homozygous State) or Cooley s Anæmia

The disease is usually diagnosed in the first year of life anæmia often becoming marked within a few weeks of birth Pallor is the predominant sign and this is accompanied by swelling of the abdomen due to splenomegaly and to a lesser extent to enlargement of the liver Overt jaundice is unusual Purpura and lymph node enlargement do not as a rule occur Bouts of pyrexia are not infrequent As in sickle-cell disease bone tenderness may be present (Smith *et al* 1955a) As the child grows widening of the cranial bone diploe may lead to enlargement of the skull and often to a mongoloid appearance Growth as a whole is often retarded and this may lead to dwarfism (de Muro and Leonardi 1950) The spleen may become greatly enlarged

Radiological examination of the child s bones typically reveals thinning of the cortical compact bone and resorption of trabeculæ Rarely spontaneous fractures occur (Ray Basu and Chatterjea 1956) The outer table of the skull may become extremely thin and the diploe greatly widened Characteristic perpendicular striæ often appear between the inner and outer tables (Cooley Witwer and Lee 1927 Baty Blackfan and Diamond 1932 Caffey 1937 1951 1957) Intractable ulcers of the leg occasionally occur (Estes Farber and Stickney 1948 March Schlyen and Schwartz 1952 Cooper and Walker 1954 Pascher and Keen 1957) Pascher and Keen suggested that ulceration results from a combination of the effect of stasis long continued anæmia trauma and some as yet unknown factor All the patients they reviewed were adolescents or adults (ages 14–32 years) Gall stones have been recorded (Currin and Lieberman 1951 Smith and Morgenthau 1951)

Astaldi Tolentino and Sacchetti (1951a) referred to three grades of thalassæmia major (1) a severe form causing serious anæmia early in infancy and often resulting in death in the first year (2) a slightly less severe form of the disease usually first becoming manifest in the second half of the first year the child often surviving until school age and (3) a milder form usually diagnosed in the second year of life and compatible

increased fertility and a reduced susceptibility to malaria (Neel 1956–57 Carcassi Ceppellini and Pitzus 1957)

Banton (1951) Ludwin Limentani and Dameshek (1952) and Vulpis (1955) failed to demonstrate any linkage between the gene for Mediterranean anæmia and those for the ABO and Rh blood groups and eye colour

Racial Incidence Thalassæmia is usually considered to affect principally people living in the Central and Eastern Mediterranean area Thus the disease is common in Italians Sardinians Sicilians Greeks (see Fessas 1959b) and Cypriots but it also exists elsewhere in the Mediterranean islands and littoral *e g* in Turks and *Eti Turks (Aksoy Ikin Mourant and Lehmann 1958 Aksoy*, 1959) In recent years however there has been an increasing awareness of the presence of Mediterranean anæmia in many countries throughout the world and in many races (Silver 1950 March Schlyen and Schwartz 1952) (Fig 83) Particularly is this true of the Far East (Brumpt 1955) Whether this incidence represents a spread of the gene throughout the world as the result of ancient and modern migrations of Mediterranean peoples the Mediterranean area being the site of the original focus or whether there have been several foci of origin is uncertain Brumpt (1955) suggested that the disease may in fact have originated in China and suggested Sinémie as an alternative to 'thalassémie' It is also uncertain as to whether or not thalassæmia is homogeneous The probability is that closely allied disorders exist This is discussed on p 227

Some of the more important descriptions of thalassæmia' in non Mediterranean populations are listed below In most instances it is difficult to exclude the possibility of remote Mediterranean ancestors Most of the published accounts refer to thalassæmia minor

Cases have been described in persons of English or Scottish ancestry (Bywaters 1948 Israels Suderman and Hoogstraten 1955 Israels and Turner 1955 Havard Lehmann and Bodley Scott 1958) and the author is aware of several other (unpublished) families living in London and the Home Counties in Bantu living in the Belgian Congo (Stijns and Charles 1956) in Bulgarians (Panoff 1936) in Burmese (Perabo 1954) in Ceylonese (de Silva Jonxis and Wickramasinghe 1959) in *Chinese (Foster 1940 DeMarsh 1950) in* Chinese Canadians (Sidoo Coady Morgan Dean and Perry 1956) in Filipinos (Lazar 1956) in Germans (Heilmeyer Muller and Schubothe 1951 Pribilla 1951 Middlebrook 1956) in Indians (Hindus and Sikhs) (Napier Shorten and Das Gupta 1939 Dhayagude 1944 Ganguli and Lahiri 1955 Sidoo *et al* 1956 Chatterjea 1959) in Indonesians (Lie Injo Luan Eng and Tjay 1955 Lie Injo Luan Eng 1959) in Iranians (Nuyken

Table 11

Blood Counts and Other Hæmatological Data of a Child (H M) Suffering from Severe Mediterranean Anæmia (Thalassæmia Major) and of his Parents both of whom were Carriers of the Mediterranean Anæmia Trait (Thalassæmia Minima)

Patient	Erythrocytes (millions per cu mm)	Hæmoglobin (g per 100 ml)	MCV (cu μ)	MCHC (%)	Reticulocytes (%)	Normoblasts (per cu mm)	Serum bilirubin (mg per 100 ml)	Fœtal hæmoglobin (%)
H M (aged 3)	4 1	5 0	54	23	8 4	8 000	1 2	12–20
Father of H M	6 5	14 1	74	20	2 4	0		0
Mother of H M	5 4	12 7	77	31	2 2	0		0

with survival until adult life Bone lesions were particularly conspicuous in patients belonging to the second and third groups

The literature dealing with the occurrence of thalassæmia major in patients surviving until adult life was reviewed by March Schlyen and Schwartz (1952) who added two more cases of their own Fessas (1959a) gives further details of adult patients seen in Greece apparently homozygous for thalassæmia whose illness was of intermediate clinical severity

Blood Picture Anæmia is generally severe the erythrocyte count lying as a rule between 1 000 000 and 3 000 000 cells per cu mm The cells vary greatly in size and shape both microcytes and macrocytes being present and many are unusually flattened (Baty Blackfan and Diamond 1932 Bradford and Dye 1936) In stained films the appearances are those of severe hæmoglobin deficiency and most but not all of the corpuscles stain palely (Fig 85) Some cells appear as rings of hæmoglobin with little or no staining in the middle other cells are target cells still others are mere fragments irregular in contour—these stain as a rule relatively densely Normoblasts are almost invariably present the greatest numbers being found in the most severely affected cases Some of the normoblasts are early forms in others the nucleus is pyknotic and the cytoplasm apparently ripened A moderate degree of polychromasia and punctate basophilia is usually seen The reticulocyte count is usually above normal and may reach 10% or even more Bradford and Dye recorded the mean corpuscular diameter (MCD) in eight patients as ranging from 5 8 to 7 4 μ After splenectomy in two patients the MCD was greater than normal 8 0–8 3 μ and 7 5–7 7 μ respectively

The leucocyte count is usually raised and may even exceed 25 000 cells per cu mm A small percentage of myelocytes is commonly found The platelet count is generally normal

Data obtained from a child aged 3 years suffering from the disease in its typical form and from the child s parents are given in Table 11

Osmotic Fragility of the Erythrocytes This is characteristically abnormal The resistance to hæmolysis of the majority of the patient s corpuscles is increased but there may be in addition a small percentage of abnormally fragile cells (Baty Blackfan and Diamond 1932) (Fig 84) Hæmolysis is often incomplete in 0 2% saline and sometimes even in 0 1% saline

The changes resulting from the incubation at 37 C of the blood of a patient with severe Mediterranean anæmia were studied by Selwyn (1953) He found (*a*) that the rate of autohæmolysis was at the upper limit of normal (0 8% at 24 hours and 3 3% at 48

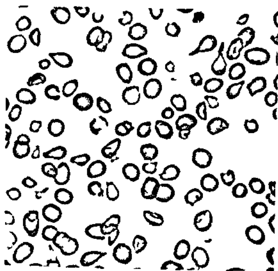
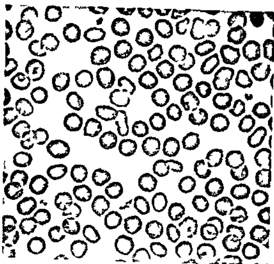


Fig 8 Pl ot microgr]h of a blood flm of a chil i suff r ng from Mediterr n an *anæmia (tl al* a *sem* a major) There is con ji uous m croeytosi an l evilence of fragm ntati n of tl erythrocyte 00



F 8(Photom c *ograt} of a Il ɔl* flm of a carrier of the Med terr nean anæmia trait (tl ala em a m ma) × 700

hours) (*b*) that the cell volume diminished instead of increasing as is normal (*c*) that the loss of potassium from the corpuscles was greater than normal, and (*d*) that the erythrocyte osmotic fragility was markedly diminished rather than increased as the result of the 24 hours incubation Thus there is some evidence that the Mediterranean anæmia erythrocyte behaves abnormally on incubation as well as being morphologically abnormal

Mechanical Fragility There is some doubt as to whether this is normal or abnormal in thalassæmia major According to Tolentino

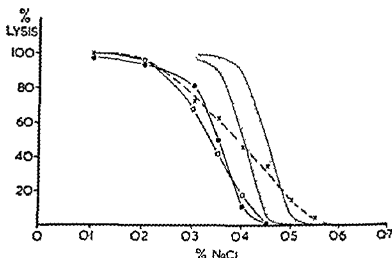


FIG 84 Erythrocyte osmotic fragility curves of the blood of a child suffering from severe Mediterranean anæmia (thalassæmia major) x – – – – – x and of his parents both of whom were carriers of the Mediterranean anæmia trait (thalassæmia minima) ● ——— ● and o ——— o The shaded area represents the normal range

(1951) mechanical fragility is normal or even slightly diminished Chatterjea Ghosh Ray and Das Gupta (1950) and Das Gupta and co-workers (1958) however have reported slight increases in Cooley's anæmia and normal findings in Cooley's trait (thalassæmia minor)

The *bone marrow findings* in thalassæmia are described on p 212

Thalassæmia Minor and Minima (Heterozygous State)

The symptoms produced by the disease in the heterozygous state are far less serious than in homozygotes and most patients are capable of leading moderately active lives (Wintrobe *et al*

1940 Strauss Daland and Fox 1941 Dameshek 1943) In the mildest cases there may be no complaints attributable to the disease although hæmatological examination reveals definite abnormalities (thalassæmia minima or the microcytemia of Silvestroni and Bianco (1946)) In the less fortunate patients the disease results in chronic anæmia of mild to moderate degree and in these cases vague tiredness and dyspnœa on exertion are common complaints (thalassæmia minor) In some patients chronic jaundice of acholuric type is a feature (Rietti Greppi Micheli disease of Italian authors) The spleen is generally palpable in the moderately severely affected patient and ulcers of the leg and gall stones have been observed (Marmont and Bianchi 1948) X ray studies may reveal some degree of osteoporosis and certain physical stigmata such as broadening of the nose and prominence of the cheek bones may also be present

Occasionally patients heterozygous for the thalassæmia gene may be quite seriously ill and moderately severely anæmic *e g* their hæmoglobin may be as low as 7 5 g per 100 ml Zuelzer Neel and Robinson (1956) pointed out that the use of the word minor to describe such cases is probably a misnomer Some of these intermediate cases (Sturgeon Itano and Bergren 1955) may be combinations of thalassæmia with abnormal hæmoglobins *e g* hæmoglobin E but this does not seem to be the explanation in every case Clinically a severe thalassæmia minor may approach unusually benign homozygous thalassæmia major in severity In the present author s opinion there is much to be said for using the term thalassæmia minor to describe patients who are heterozygous for thalassæmia and who have the disease in a clinically significant degree and to reserve the term thalassæmia trait (or thalassæmia minima) for heterozygotes who are entirely symptom free but who have nevertheless detectable hæmatological abnormalities such as microcytosis and increased osmotic resistance The two groups in practice merge one into the other

Blood Picture Most patients have a mild to moderate anæmia with the hæmoglobin level not usually reduced below 10 g per 100 ml However in the minima state the hæmoglobin level is usually normal Erythrocyte counts are less reduced often they are within the normal range not infrequently they exceed 6 000 000 cells per cu mm (Wintrobe *et al* 1940 etc) (Table 11) Twenty two out of 80 cases reported by Hanlon and Bayrd (1956) had counts exceeding 5 000 000 cells per cu mm The proportion of reticulocytes is generally above normal but seldom exceeds 5% (Smith 1943 Valentine and Neel 1944 1948 Hanlon and Bayrd 1956) Valentine and Neel (1948) studied 82 affected subjects and compared the findings with those of the normal members of the family groups They found that the differences between affected

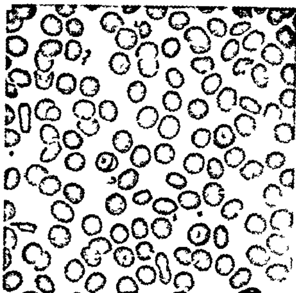


FIG 87 Photomicrograph of a blood film of a carrier of the Mediterranean anæmia trait (thalassæmia minima) Target cells are unusually conspicuous × 700

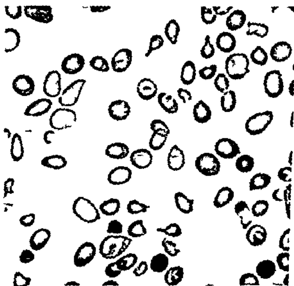


FIG 88 Photomicrograph of a blood film of a patient suffering from an intermediate grade of Mediterranean anæmia complicated by folic-acid deficiency Oval macrocytes are conspicuous × 700

Schwartz, 1954) but this is not necessarily so (Crosby and Sacks 1949 Chanarin Dacie and Mollin 1959) Fessas (1959a) similarly refers briefly to two patients (adults with thalassæmia major of intermediate severity) who responded dramatically to folic acid and also mentions that aplastic crises are common during intercurrent infections in adult patients

Folic acid deficiency leads to the blood film of a patient with thalassæmia minor becoming more than usually bizarre Oval macrocytes (Fig 88) increased poikilocytosis fragmentation and multisegmented neutrophils together form a suggestive picture It is important to recognize such cases as marrow failure due to this cause may result in a crisis in a patient whose anæmia is normally well compensated (The recognition of megaloblastic change in the marrow is however less easy than in the absence of thalassæmia. Typical megaloblasts may not be visible Erythropoiesis however becomes more immature in type and giant myelocytes may be present)(*cf* Figs 89 and 90)

Biochemical Findings

Hæmoglobin Type Abnormal hæmoglobins are typically not present in Mediterranean anæmia of either the major or minor varieties The abnormal persistance of Hb F is however a characteristic feature. This is discussed in detail on p 224

Erythrocyte Porphyrins Sturgeon Chen and Bergren (1958) have recently reported on their findings in seven patients with thalassæmia major and ten with thalassæmia minor In the major disease the concentrations of coproporphyrin and uroporphyrin were greatly increased and that of protoporphyrin was just at the upper limit of the normal range In thalassæmia minor the concentrations were normal Sturgeon Chen and Bergren point out however that similar high concentrations of coproporphyrin and uroporphyrin may be found in other types of hæmolytic anæmia associated with a raised reticulocyte count

Serum Bilirubin This is usually within the range 1–3 mg per 100 ml in thalassæmia major in thalassæmia minor or minima the concentration is usually normal but may be raised in cases of the Rietti Greppi Micheli type

Serum Iron The concentration is characteristically higher than normal in thalassæmia major and may be normal or slightly higher than normal in thalassæmia minor (Cartwright *et al* 1948) the iron binding capacity of the serum may be fully saturated in the major form of the disease (Smith Sisson Floyd and Siegal 1950) Serum copper levels are normal or increased (Cartwright *et al* 1948)

Plasma Hæmoglobin This has been estimated by Crosby and Dameshek (1951) and Das Gupta Chatterjea Ray and Ghosh (1955) In 23 patients with thalassæmia minor Crosby and Dameshek found that the plasma hæmoglobin was normal (1–3 mg per 100 ml) in three out of four patients with severe Mediterranean anæmia (probably thalassæmia major) it was elevated (12–60 mg per 100 ml) while in the fourth patient the level was normal before splenectomy but rose to

and non affected siblings was well established from childhood onwards On the whole affected females were more anæmic than affected males

Erythrocyte Morphology Definite abnormalities in erythrocyte morphology are probably always to be found if sought for even in patients without anæmia or where there is erythrocytosis The erythrocytes vary more than normally in size The MCV is usually well below normal (Smith 1943 Valentine and Neel 1944 1948 Heinle and Read 1948 Daland and Strauss 1948 Gerald and Diamond 1958a) The MCD on the other hand is generally within the normal range (Mooney 1952) the presence of some macrocytes balancing the effect of numbers of microcytes Characteristically the mean cell thickness (MCT) is considerably reduced (leptocytosis) (Dameshek 1940 Wintrobe *et al* 1940 Smith 1943)

The erythrocytes stain palely with Romanowsky dyes this is mostly due to their diminished thickness as the hæmoglobin concentration as a rule is only slightly reduced and may be normal (Smith 1943 Valentine and Neel 1944 Daland and Strauss 1948 Heinle and Read 1948 Gerald and Diamond 1958a) A ring type of staining (Wintrobe *et al* 1940) is characteristic in some cases target cells are present (Figs 86 and 87) but not usually in the large numbers associated with the presence of Hb C Often many of the erythrocytes are moderately oval in shape (Dameshek 1940) Punctate basophilia is sometimes conspicuous (Wintrobe *et al* 1940 Smith 1948 Rietti 1950 Mooney 1952 Hanlon and Bayrd 1956) On the whole the morphological changes are far less severe and the erythrocytes are more uniform in appearance than in the major form of the disease On the other hand the abnormalities are relatively severe in relation to the mildness of the anæmia that may be present Normoblasts and myelocytes are not usually present in the peripheral blood

Osmotic Fragility Characteristically the resistance to hypotonic saline is markedly increased and this is found to be present to some extent even in the absence of anæmia (Smith 1943 Valentine and Neel 1944 Mooney 1952 Gatto and Valentino 1953 Hanlon and Bayrd 1956) (Fig 84)

A Complication of Thalassæmia Minor Severe Anæmia due to Folic Acid Deficiency

Folic acid deficiency has been recently recognized as a rare complication of thalassæmia (see Chanarin Dacie and Mollin 1959) Pregnancy may be an important contributory factor (Goldberg and



FIG 89 Photomicrograph of sternal marrow film of a patient with an intermediate grade of Mediterranean anæmia complicated by folic acid deficiency Erythropoiesis is megaloblastic See also Figs 88 and 90 May Grunwald Giemsa × 1100 (From Chanarin Dacie and Mollin (1959))

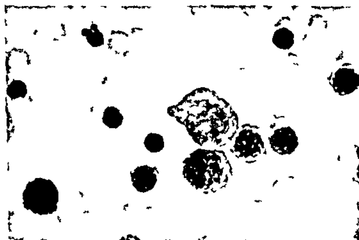


FIG 90 Photomicrograph of a sternal marrow film of a patient with an intermediate grade of Mediterranean anæmia complicated by folic acid deficiency After treatment with folic acid (cf Fig 89) May Grunwald Giemsa × 1100 (From Chanarin Dacie and Mollin (1959))

25 mg per 100 ml after splenectomy Das Gupta and his colleagues reported essentially similar findings 13 patients with the Cooley trait had values of 0 6–4 3 mg per 100 ml and 22 patients with Cooley s anæmia values of 4 3–56 5 mg per 100 ml Substantially similar figures based on a larger series of patients were given by Das Gupta and his co workers (1958)

Serum Proteins There were studied using paper electrophoresis by Allamanis (1955) in 36 children with Cooley s anæmia in 11 infants less than 24 months old the patterns were almost normal in older children albumin was decreased (on an average by 52%) and γ globulin increased (on an average by 30%) there were minor reductions in the concentrations of α_1 α_2 and β globulins and the flocculation tests often gave abnormal results

Increases in the plasma and corpuscular lipids and phospholipids were described by Erickson and co workers (1937) and Schwarz Tiene Corda and Careddu (1953) the latter authors also reporting low levels of free and combined cholesterol Recently Choremis Zannos and Basti (1957) have reported finding in 20 infants and children with Cooley s anæmia that the output of amino acids in the urine was markedly increased The significance and cause of this abnormality are obscure

Urobilinogen Excretion This is increased and as in pernicious anæmia it is probable that not all the fæcal urobilinogen is derived from erythrocytes which have circulated in the blood stream Sturgeon and Finch (1957) observed excretions of 400–600 mg per day in three children which were about ten times the anticipated amount

Pathology

Bone Marrow in Thalassæmia The bone marrow is hyperplastic the degree of hyperplasia varying directly with the severity of the anæmia The hyperplasia is the result of active erythropoiesis and in severe cases the erythroid myeloid ratio may exceed unity Erythropoiesis is typically normoblastic There is a tendency for the developing normoblasts to be smaller than normal (micronormoblasts) (Fig 90) this is mostly due to diminution in the amount of cytoplasm and is most marked in the most ripened cells Detailed measurements are given by Astaldi Tolentino and Sacchetti (1951a) In thalassæmia major the percentage of basophilic normoblasts is often unusually high in thalassæmia minor polychromatic and pyknotic normoblasts predominate Astaldi and Tolentino (1952) claimed that in the most serious cases of thalassæmia major there was some delay in the enucleation of the orthochromatic normoblasts Megaloblastic change as the result of folic acid deficiency has already been referred to as rather a rare event (Fig 89)

In severe cases hæmoglobin may appear to be formed in a patchy fashion in the cytoplasm of the developing normoblasts

areas of eosinophilic hæmoglobin being interspersed between remnants of the primitive basophilic cytoplasm (Fig 91) This gives rise to an appearance of rather coarse and irregular punctate basophilia

In striking contrast to the finding in simple iron deficiency siderotic granules are present in the marrow normoblasts (Douglas and Dacie 1953) Typically they are more numerous and larger than in health (Fig 92)

Other Organs *Spleen* The spleen is usually markedly enlarged Sections show congestion extramedullary hæmopoiesis and a thickened reticulum Deviation from the normal is far less marked in thalassæmia minor than in the major disease The amount of iron present depends upon the number of times if any the patient has received blood transfusions In the absence of a history of transfusions only relatively small amounts of iron are present in the spleen

Liver The iron content of the liver is moderately increased even in the absence of transfusions and the same is apparently true of the iron content of the kidneys heart pancreas and lymph nodes etc (Whipple and Bradford 1936 Astaldi Tolentino and Sacchetti 1951)

More recent papers giving detailed histo pathological findings include those of Sansone Rosso Zunin and Salomone (1955) and Fornara and Genesi (1955) Howell and Wyatt (1953) and Ellis Schulman and Smith (1954) reported careful studies on patients who had developed fibrosis of the liver and other organs and they discussed the relationship between their findings and those of idiopathic hæmochromatosis and transfusional siderosis There appeared to be no correlation between fibrosis siderosis and the degree and chronicity of the anæmia In some cases the amount of iron present exceeded that which could be accounted for by transfusion and it seemed probable that the amount absorbed from the gastro intestinal tract might have been unusually large (possibly due to the chronic anæmia)

As in other congenital hæmolytic anæmias extramedullary foci of erythropoiesis are sometimes found These foci may even give rise to symptoms for instance Gatto Terrana and Biondi (1954) reported that an epidural mass of marrow caused compression of the spinal cord in one of their patients

Diagnosis of Mediterranean Anæmia

Thalassæmia Major The disease is diagnosed from a consideration of the clinical and hæmatological data and from family studies The rather variable clinical form of the major disease has already been referred to (p 205) In the most severe type affecting infants with many primitive erythroblasts



FIG. 91. Photomicrograph of a sternal marrow film of a child with severe Mediterranean anaemia (thalassaemia *major*) showing irregular formation of haemoglobin and punctate basophilia in the cytoplasm of normoblasts. A rounded mass of haemoglobin is marked with an arrow. May-Grünwald-Giemsa. × 1100.

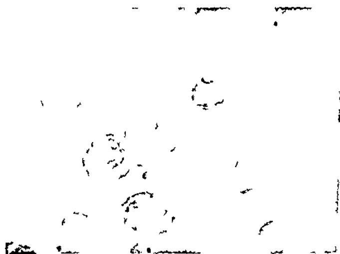


FIG. 92. Photomicrograph of a sternal marrow film of a child aged 3½ years with a severe grade of Mediterranean anaemia (thalassaemia *major*) showing iron-containing granules in normoblasts. Perls's acid ferrocyanide reaction. × 1100.
The child had been transfused with approximately 5 litres of packed erythrocytes during the preceding 3 years.

(which are absent in iron deficiency) the response to treatment with iron and the results of family studies are usually decisive in diagnosis

The presence of easily detectable amounts of fœtal hæmoglobin is in favour of thalassæmia minor and against the diagnosis of iron deficiency However the presence of fœtal hæmoglobin should not in the absence of other supporting evidence be taken as necessarily indicating Mediterranean anæmia According to recent observations (see p 267) fœtal hæmoglobin occasionally persists into adult life without being associated with any other hæmatological abnormality Its persistence appears to be controlled by an abnormal gene which is probably distinct from that of typical thalassæmia Small amounts of fœtal hæmoglobin have also been detected although rarely in other types of hereditary hæmolytic anæmia *e g* in hereditary elliptocytosis (p 154)

Recently use had been made of starch block electrophoresis in the differentiation of thalassæmia from other conditions The A_2 component of hæmoglobin (Kunkel and Wallenius 1955) is apparently present in characteristically high concentrations in thalassæmia minor (Kunkel Ceppellini Muller Eberhard and Wolf 1957 Gerald and Diamond 1958a Josephsen *et al* 1958) (see also p 226) In iron deficiency anæmia the Hb A_2 content is lower than normal (Josephson *et al* 1958)

As already mentioned patients with the mildest forms of thalassæmia may have abnormally high erythrocyte counts This type of blood picture may be confused with other forms of polycythæmia In thalassæmia minima the hæmoglobin levels will be found to be normal or subnormal the erythrocytes hypochromic and microcytic and the leucocyte and platelet counts normal Similarly blood containing many conspicuously oval or elliptical erythrocytes may be confused with that of hereditary elliptocytosis In the latter disorder the degree of elliptocytosis is usually more pronounced and regular and the number of cells affected by the change as a rule much greater than in thalassæmia The cells in true elliptocytosis are normochromic not hypochromic and have a normal not reduced MCV and poikilocytosis is much less conspicuous than in Mediterranean anæmia

Treatment of Mediterranean Anæmia

Nothing has yet been found that will produce a sustained favourable effect on erythropoiesis Iron therapy either by mouth

in the peripheral blood confusion with erythræmic myelosis (di Guglielmo s disease) may arise However in the latter disorder myeloblasts will probably be found in quite large numbers in the peripheral blood—these are usually absent in Mediterranean anæmia The erythrocytes too will not be conspicuously hypochromic Knowledge of the family history and of the blood picture in relatives also helps in arriving at the correct diagnosis

In the less severe forms occurring in childhood the clinical history blood picture and other hæmatological findings are generally characteristic and there should be no real difficulty in diagnosis The same applies to the few patients who reach adult life

Electrophoretic studies of the patient s hæmoglobin play an essential part in the differentiation of Mediterranean anæmia from the hæmoglobinopathies and mixed syndromes such as Hb S/thalassæmia etc As already mentioned abnormal hæmo globins are *not* found in pure thalassæmia although a high concentration of fœtal hæmoglobin (Hb F) is characteristic (p 224)

Thalassæmia major can be distinguished from severe iron deficiency anæmia by the more severe changes in the erythrocytes in the former disease by the presence of large amounts of Hb F in thalassæmia major by study of the serum iron level or iron content of the bone marrow—low in iron deficiency anæmia high in thalassæmia and by the fact that the patient suffering from thalassæmia fails to benefit from intensive treatment with iron

Thalassæmia Minor and Minima Here the separation from iron deficiency anæmia is less easy On clinical grounds the facies of the patient his history the degree of enlargement of the spleen and the possible slight jaundice all point away from iron deficiency as the cause of the anæmia It may be difficult to decide on the blood picture alone hypochromasia anisocytosis and the presence of oval cells and elliptocytes target cells and punctate basophilia —characteristic features of thalassæmia minor may all be found in true iron deficiency anæmia to a greater or lesser degree The same applies to increased erythrocyte osmotic resistance However it is probably true that the numbers of target cells and cells showing punctate basophilia are less likely to be as high and the diminution is osmotic resistance is less likely to be as severe (Reimann and Arkun 1955) in simple iron deficiency anæmia as compared with Mediterranean anæmia Once again knowledge of the serum iron level and bone marrow content of iron—in particular the presence in the cytoplasm of normoblasts in thalassæmia of granules of iron demonstrable by Perls s reaction

transfusions to attempt to raise the hæmoglobin much above 10 g per 100 ml

Splenectomy The spleen has often been removed and until recently the general consensus of opinion seems to have been that this ordinarily makes little difference to the course of the disease It is interesting to recall however the views of Penberthy and Cooley (1935) They stated The organ becomes so large however that it seems worthwhile to remove it there is no particular change in the anemia after the operation We have felt so far as our cases are concerned that the patients were more comfortable and lived somewhat longer after splenectomy than we would have expected without the operation. We have operated on five patients We believe that it is worth doing

It is now realized that splenectomy is likely to be of most benefit in patients in whom hæmolysis is marked (Govan 1946 Chini and Valeri 1949 Lichtman *et al* 1953 Gatto and Lo Jacono 1953 Minnich *et al* 1954 Glenn *et al* 1954 Smith *et al* 1955a Greppi and di Guglielmo 1955 Reemtsma and Elliott 1956 Clement and Taffel 1955 Rapaport Reilly and Carpenter 1957 Mainzer and O Connor 1958)

Lichtman and co-workers observed in children suffering from thalassæmia major who had received repeated transfusions that it was quite common for the transfusions to be required at increasingly frequent intervals if the patients hæmoglobin levels were to be maintained They studied by means of the Ashby method the fate of the blood transfused to seven children and found a shortened survival in each case in six of them the half life of the transfused cells was reduced to between 5 and 9 days and in the seventh child it was 35 days This suggested a superadded extracorpuscular mechanism of cell destruction However no abnormal antibodies the presence of which might have explained these findings could be identified Splenectomy was carried out in five of the patients in four of them the volumes of blood required to be transfused after splenectomy were reduced to 19 21 28 and 36 % respectively of the volumes necessary before splenectomy It was concluded that a good case could be made out for removal of the spleen when transfusion studies indicated an abnormal rate of erythrocyte destruction. This seemed likely to occur most commonly in patients in whom the spleen was greatly enlarged

The observations and conclusions of Lichtman and co-workers have been largely confirmed in other centres The best results have been obtained in older children with large spleens in whom there was evidence of subnormal survival of transfused normal erythrocytes or other evidence of hypersplenism such as leucopenia or thrombocytopenia

Glenn and co-workers (1954) and Smith and co-workers (1955a) reported on the results of splenectomy in nine cases of thalassæmia. All the patients had improved hæmoglobin levels subsequently and

or intravenously is useless and may be dangerous—indeed the serum iron concentration is usually raised and the storage organs of the body also contain an excess of iron All the vitamin preparations that have been tried seem to be valueless [1] It is possible that cobalt may be of slight value Berk Burchenal and Castle (1949) reported rather doubtful improvement in one patient and other instances of possible benefit have been referred to by Wiessbecker (1951) Muratore (1951) and Heilmeyer Muller and Schubothe (1951) Virdis (1952) on the other hand did not observe any improvement in six children given 20 mg of cobaltous chloride orally for 10–20 days Its use can hardly be recommended with any enthusiasm

Blood Transfusion This has no fundamental effect on the course of the disease However normal erythrocytes survive normally in uncomplicated Mediterranean anæmia and for this reason great temporary benefit can be expected to result from transfusion (Hamilton Sheets and DeGowin 1950 Frontali and Stegagno 1951 Murano 1955) The improvement in general well being is striking the temper of the child improves growth takes place more normally bone pains are alleviated and in a child receiving regular transfusions the X ray evidence of an abnormal bony pattern becomes less marked

Repeated transfusions will in time lead to marked siderosis but this in the author s opinion should not be used as an argument *against periodic transfusion in patients in whom without* transfusion the degree of anæmia leads to serious symptoms Frumin Waldman and Morris (1952) referred to a child who died aged 10 years having received over 76 litres of blood since birth *At necropsy there were all the signs of exogenous hæmochromatosis* with early active portal cirrhosis of the liver (see also Howell and Wyatt (1953) and Ellis Schulman and Smith (1954)) Fortunately however most affected children manage to accommodate themselves remarkably well to hæmoglobin levels even as low as 5 g per 100 ml if this is so they are best left untransfused

Careful studies have been carried out on the effect that transfusion has on the formation of erythrocytes by the recipient As anæmia is corrected the output of erythrocytes diminishes This can be demonstrated by the technique of differential agglutination and by using the presence of fœtal hæmoglobin as a marker of the patient s own corpuscles (Smith *et al* 1955b Murano 1955) Because transfusion has an inhibitory effect on erythropoiesis it is probably unwise in a child receiving repeated

[1] With the possible exception of folic acid in rare instances (see p 211)

transfusions to attempt to raise the hæmoglobin much above 10 g per 100 ml

Splenectomy The spleen has often been removed and until recently the general consensus of opinion seems to have been that this ordinarily makes little difference to the course of the disease It is interesting to recall however the views of Penberthy and Cooley (1935) They stated The organ becomes so large however that it seems worthwhile to remove it there is no particular change in the anemia after the operation We have felt so far as our cases are concerned that the patients were more comfortable and lived somewhat longer after splenectomy than we would have expected without the operation We have operated on five patients We believe that it is worth doing

It is now realized that splenectomy is likely to be of most benefit in patients in whom hæmolysis is marked (Govan 1946 Chini and Valeri 1949 Lichtman *et al* 1953 Gatto and Lo Jacono 1953 Minnich *et al* 1954 Glenn *et al* 1954 Smith *et al* 1955a Greppi and di Guglielmo 1955 Reemtsma and Elliott 1956 Clement and Taffel 1955 Rapaport Reilly and Carpenter 1957 Mainzer and O Connor 1958)

Lichtman and co workers observed in children suffering from thalassæmia major who had received repeated transfusions that it was quite common for the transfusions to be required at increasingly frequent intervals if the patients hæmoglobin levels were to be maintained They studied by means of the Ashby method the fate of the blood transfused to seven children and found a shortened survival in each case in six of them the half life of the transfused cells was reduced to between 5 and 9 days and in the seventh child it was 35 days This suggested a superadded extracorpuscular mechanism of cell destruction However no abnormal antibodies the presence of which might have explained these findings could be identified Splenectomy was carried out in five of the patients in four of them the volumes of blood required to be transfused after splenectomy were reduced to 10 21 28 and 36 ′ respectively of the volumes necessary before splenectomy It was concluded that a good case could be made out for removal of the spleen when transfusion studies indicated an abnormal rate of erythrocyte destruction This seemed likely to occur most commonly in patients in whom the spleen was greatly enlarged

The observations and conclusions of Lichtman and co-workers have been largely confirmed in other centres The best results have been obtained in older children with large spleens in whom there was evidence of subnormal survival of transfused normal erythrocytes or other evidence of hypersplenism such as leucopenia or thrombocytopenia

Glenn and co workers (1954) and Smith and co workers (1955a) reported on the results of splenectomy in nine cases of thalassæmia All the patients had improved hæmoglobin levels subsequently and

or intravenously is useless and may be dangerous—indeed, the serum iron concentration is usually raised and the storage organs of the body also contain an excess of iron All the vitamin preparations that have been tried seem to be valueless [1] It is possible that cobalt may be of slight value Berk Burchenal and Castle (1949) reported rather doubtful improvement in one patient and other instances of possible benefit have been referred to by Wiessbecker (1951) Muratore (1951) and Heilmeyer Muller and Schubothe (1951) Virdis (1952) on the other hand did not observe any improvement in six children given 20 mg of cobaltous chloride orally for 10–20 days Its use can hardly be recommended with any enthusiasm

Blood Transfusion This has no fundamental effect on the course of the disease However normal erythrocytes survive normally in uncomplicated Mediterranean anæmia and for this reason great temporary benefit can be expected to result from transfusion (Hamilton Sheets and DeGowin 1950 Frontali and Stegagno 1951 Murano 1955) The improvement in general well being is striking the temper of the child improves growth takes place more normally bone pains are alleviated and in a child receiving regular transfusions the X ray evidence of an abnormal bony pattern becomes less marked

Repeated transfusions will in time lead to marked siderosis but this in the author s opinion should not be used as an argument against periodic transfusion in patients in whom without transfusion the degree of anæmia leads to serious symptoms Frumin Waldman and Morris (1952) referred to a child who died aged 10 years having received over 76 litres of blood since birth At necropsy there were all the signs of exogenous hæmochromatosis with early active portal cirrhosis of the liver (see also Howell and Wyatt (1953) and Ellis Schulman and Smith (1954)) Fortunately however most affected children manage to accommodate themselves remarkably well to hæmoglobin levels even as low as 5 g per 100 ml if this is so they are best left untransfused

Careful studies have been carried out on the effect that transfusion has on the formation of erythrocytes by the recipient As anæmia is corrected the output of erythrocytes diminishes This can be demonstrated by the technique of differential agglutination and by using the presence of fœtal hæmoglobin as a marker of the patient s own corpuscles (Smith *et al* 1955b Murano 1955) Because transfusion has an inhibitory effect on erythropoiesis it is probably unwise in a child receiving repeated

[1] With the possible exception of folic acid in rare instances (see p 211)

characteristic of the disease. In all except the mildest type of the disease hæmoglobin formation is inadequate and the patient fails to maintain a normal hæmoglobin level despite the hyperplasia of his erythropoietic tissue.

Crosby and Akeroyd (1952) estimated in the three cases they studied that the hæmoglobin production amounted to only 0.18–0.30 g. per kg. per day, as compared with the normal maximum of 0.6–0.65 g. per kg. per day. Norris, Hanson and Loeffler (1956) used ^{59}Fe in studying an adult negro with moderately severe Mediterranean anæmia and found a rapid plasma clearance but poor incorporation of the iron in erythrocytes. Sturgeon and Finch (1957), in elaborate isotope studies on four children with clinically severe or intermediate Cooley's anæmia, concluded that the delivery of erythrocytes was reduced to 50% of the normal in the mildly anæmic patient and in the severely anæmic patient to as little as 15% of the normal. They considered that the defect was not in total hæmoglobin synthesis but in the fabrication of circulating erythrocytes. Bailey and Prankerd (1958) in somewhat similar studies on two patients concluded that although erythrocyte production per unit volume of marrow was reduced, total production might be greater than normal because of the expanded marrow volume. Their data showed that radioactive iron appeared in the peripheral blood at about twice the normal rate.

Further studies using radioactive iron have been more recently reported. Larizza and co-workers (1958) investigated eight patients with Cooley's anæmia and three with Rietti-Greppi-Micheli anæmia. Both groups of patients handled intravenously administered ^{59}Fe in a similar way; the plasma clearance times were rapid and more iron than normal was taken up by the liver. The output of ^{59}Fe tagged cells from the marrow was markedly subnormal and slow, the peak output being delayed to the 4–6th day.

Erlandson, Schulman, Stern and Smith (1958) studied ten children all suffering from Cooley's anæmia. Erythrocyte production was calculated to be from 2.2 to 6.1 times the normal, i.e. it did not reach that attained in patients with most other types of hæmolytic anæmia. They stressed that an individual patient's degree of anæmia depended on his personal balance between erythrocyte formation and hæmolysis.

Erythrocyte Destruction. In addition to the dyshæmopoiesis there is evidence that the rate of erythrocyte destruction is increased in thalassæmia major, although usually normal in thalassæmia minor.

Kaplan and Zuelzer (1950) transfused into normal recipients the blood from three patients with severe or moderately severe Mediterranean anæmia and followed the survival of the transfused erythrocytes by the Ashby method. Between 25% and 50% of the transfused cells disappeared from the recipients' circulation in 20–30 days; later, however, the slope of elimination ran roughly parallel to the expected rate of elimination of normal corpuscles. Kaplan and Zuelzer also transfused the erythrocytes from three women carrying the Mediterranean trait into normal recipients. The patients were

all needed less blood post operatively than before The longest follow up was 51 months One patient died of encephalitis 14 months after operation and four patients developed pericarditis of obscure origin from which however they recovered (see also p 130)

Reemtsma and Elliott (1956) reported on the follow up of a relatively large series of 25 patients who were followed from 6 to 25 years or until death Thirteen of them underwent splenectomy As mentioned previously the best results were obtained in the older patients or in those in whom there were signs of hypersplenism The results in children less than 4 years of age were generally disappointing All the patients who were receiving transfusions had diminished requirements after operation but the duration of the favourable response was variable and there seemed to be a tendency to relapse Six of the splenectomized patients died within the next 10 years but there were no post operative deaths or serious complications

More recently Mainzer and O Connor (1958) have reported the results of splenectomy in 15 patients ranging in age from 18 months to 16 years There was no immediate post operative mortality and in five of eleven patients who were followed up for a relatively long period (4–8 years) the requirement of blood for transfusion appeared to be definitely decreased i e to less than 50% of the pre operative figures Mainzer and O Connor mentioned that one patient developed pneumococcal meningitis post operatively and that two others experienced pathological fractures

Splenectomy therefore seems to have a definite but restricted place in the treatment of thalassæmia major However it does not improve erythropoiesis and should perhaps only be undertaken if there is definite evidence in a child receiving repeated transfusions of an abnormal rate of destruction of the transfused erythrocytes or if there are other signs of hypersplenism

Marked erythroblastæmia the presence of many siderocytes an increase in erythrocyte aniso poikilocytosis and an increase in the number of target cells may be expected to follow splenectomy Whipple and Bradford (1936) found that the mean erythrocyte diameter was increased and the erythrocyte thickness decreased after the spleen had been removed Erythrocyte osmotic resistance is still further increased

Pathogenesis of Mediterranean Anæmia

Impaired Erythropoiesis There is little doubt but that Mediterranean anæmia is caused by a genetically determined defect of erythrocyte formation As a result abnormal thin mis shapen erythrocytes of low hæmoglobin content are produced which in severe cases at least probably survive for an unusually short time in the circulation Bone marrow hypertrophy follows as a consequence of chronic anæmia and this in time often leads to the abnormalities of the skull and other bones which are so

destruction of short lived cells (although according to Vullo and Tumioli (1958) in this respect it is less active than a normal spleen) This is in accord with the slight to moderate clinical improvement which may follow splenectomy particularly when the spleen is greatly enlarged

Studies of the survival of normal erythrocytes in patients with Cooley s anæmia have mostly given normal results However shortened survival has been noticed (McElfresh Sharpstein and Akabane 1955 Smith *et al* 1955a) and it is in these patients who possibly have hypersplenism that splenectomy may be beneficial (see p 217)

Mechanism of Hæmolysis Whipple and Bradford (1936) recognized that the erythrocytes in Mediterranean anæmia appeared to undergo fragmentation *in vitro* unusually readily This phenomenon has often been commented on subsequently

Marmont and Bianchi (1948) in describing three cases of the Rietti Greppi Micheli type reported some detailed observations They found that the fragmentation was particularly marked in supravital preparations stained with brilliant cresyl blue under these conditions dumb bell erythrocytes appearing as two spheres of hæmoglobin united by a colourless membrane seemed to represent a stage in the fragmentation process Similar cells could also be found in films of peripheral blood allowed to dry immediately after collection According to Marmont and Bianchi only in Mediterranean anæmia is erythrocyte fragmentation so striking It is interesting to note however that they add that in severe simple iron deficiency anæmia the intensity of fragmentation may be almost as great Zintl (1954) and Perosa and Dell Aquila (1955) have reported analogous observations Perosa and Dell Aquila kept citrated thalassæmic blood at 42 C and found after 5–6 hours incubation an abnormal degree of fragmentation and hæmolysis

If the erythrocytes disintegrate *in vivo* in the peripheral blood to a marked extent in Mediterranean anæmia the plasma hæmoglobin concentration would be expected to be abnormally high This has in fact been found (see p 211) The cause of the abnormal fragmentation is not known It is presumably the consequence of defective erythropoiesis

Nature of the Defect in Erythropoiesis The exact nature of the defect of erythrocyte formation has not yet been determined Bone marrow studies show that as the normoblasts grow they develop into unusually small cells which are particularly deficient in cytoplasm The changes are reminiscent of those produced by simple iron deficiency and there seems little doubt that part at least of the fundamental defect of Mediterranean anæmia is a failure of the proper and sufficient synthesis of hæmoglobin in the presence of apparently fully adequate amounts of iron It is possible that the abnormalities of erythrocyte morphology are merely the consequence of this

clinically well having erythrocyte counts of between 4 600 000 and 5 300 000 cells per cu mm and hæmoglobin levels of between 10 and 11 5 g per 100 ml The survival of their corpuscles in normal recipients was normal The fact that in the severely affected cases some of the cells appeared to be relatively rapidly destroyed while other cells were destroyed at about the normal rate indicates a marked variability within the population of erythrocytes in respect of the defect leading to rapid lysis An examination of a blood film of a severely affected patient certainly shows that there is also great variability in morphology Kaplan and Zuelzer thought that the most deformed cells were probably eliminated first but concluded that poikilocytosis *per se* was not necessarily associated with rapid elimination

Hamilton Sheets and DeGowin (1950) also reported on the survival of Mediterranean anæmia blood The erythrocytes of a subject with *thalassæmia minima* survived normally when transfused to a normal recipient but the survival of the corpuscles of a patient with a more severe form of the trait was slightly impaired (elimination complete within 85 days)

Frontali and Stegagno (1951) transfused the blood of two children severely affected with Cooley s anæmia into recipients suffering from mild anæmia not considered to be hæmolytic in origin Using the Ashby method they found that the elimination of the transfused cells was complete in 12 and 19 days respectively Frontali (1954) reported the results of further experiments The life span of the erythrocytes of five patients with thalassæmia major was estimated to be from 18–20 to 50–70 days while that of five symptomless thalassæmia trait carriers was found to be normal

More recently studies have been carried out using ⁵¹Cr Sturgeon and Finch (1957) studied three patients and found ⁵¹Cr half times of 10 9 and 7 days respectively corresponding with a calculated rate of destruction of 7–10 times the normal Bailey and Prankerd (1958) studied two adults and found evidence of two populations of cells one population had a mean life span of only a few days and the other a mean life span of about 30 days (*cf* Kaplan and Zuelzer 1950) The short lived cells appeared to be destroyed in the spleen

Vullo and Tunioli (1958) transfused the ⁵¹Cr tagged erythrocytes of seven children with thalassæmia into a variety of recipients In the patients themselves the ⁵¹Cr half times were 9–22 days (normal 25–35 days) In four instances it was possible to transfuse normal recipients (with spleens) also in each case the survival of the patient s erythrocytes was less in the normal subject than in the patient s own circulation In three further experiments the patient s cells were divided between the patient and a splenectomized but otherwise healthy subject the rates of elimination were about the same in each pair of recipients

Erlandson and co workers (1958) have also published extensive data The ⁵¹Cr half times in ten children with thalassæmia major were 6 5–19 5 days (equivalent to mean cell life spans of 10 3–39 days) The shortened survival in this series could not be correlated either with the Hb F content of the cells or with the degree of anæmia present

Role of the Spleen

As just referred to Bailey and Prankerd (1958) by in vivo counting with ⁵¹Cr concluded that the spleen was an important organ in the

illustrations also show that some of the erythrocytes are honeycombed with vacuoles In a later paper Bessis Alagille and Breton Gorius (1958) mentioned in addition to the iron containing granules (ferratin or micelles ferrugineuses) and the vacuoles the occurrence of masses of amorphous material (of unknown nature) and of denser spherules which they believe may be the P AS positive material

Tishkoff (1958) provided quantitative data on the excessive amounts of stromal (non hæmoglobin) iron in the thalassæmic erythrocyte In labelling experiments using ^{59}Fe it could be clearly shown that more iron was actually present in cell stroma than in the hæmoglobin the cells contained

The studies mentioned in the preceding paragraphs and the fact that the bone marrow forms erythrocytes of low hæmoglobin content although the parent erythroblasts contain an excess of iron containing granules visible by ordinary microscopy strongly support the hypothesis that hæmoglobin synthesis is impaired in Mediterranean anæmia Unfortunately they do not provide evidence as to how and why this happens It is clearly a problem which can only be solved by biochemical techniques A step towards a solution has been provided by the recent elaborate study of Bannerman Grinstein and Moore (1959) They measured the incorporation of [2 ^{14}C]glycine and ^{59}Fe by the immature cell population of peripheral blood samples from nine patients with thalassæmia major seven of whom had undergone splenectomy Although the results varied widely between the samples the hæm synthetic activity was generally low when related to the numbers of immature cells present However the thalassæmia samples showed a brisk incorporation of [^{14}C]protoporphyrin into hæm and the incorporation of ^{59}Fe into hæm in a thalassæmia sample was greatly increased by the addition of free protoporphyrin No abnormality in globin synthesis could be demonstrated and it was tentatively concluded that hæm synthesis was impaired in two ways (1) the rate of protoporphyrin synthesis was abnormally slow and (2) there was a partial block in the combination of protoporphyrin and iron

Hæmoglobin in Mediterranean Anæmia

As already indicated an important difference between Mediterranean anæmia and iron deficiency anæmia lies in the fact that a relatively large amount of the hæmoglobin in thalassæmia major is of the fœtal (Hb F) type The first relevant observations were made by Vecchio (1946 1948) and Putignago and Fiore Donati (1948) who showed that the rate of alkali denaturation was slowed This work was soon confirmed and other points of

However certain features suggest that a defect in hæmoglobin synthesis may not be the whole extent of the abnormality of erythropoiesis For example the abnormalities in the erythrocytes in thalassæmia major are more severe than are seen in simple iron deficiency anæmia in severe cases too there may be an actual defect in the maturation of normoblasts (Hamilton and Fowler 1951 Astaldi Tolentino and Sacchetti 1951b Astaldi and Tolentino 1952) On the other hand it can be argued that the remarkable changes in erythrocyte morphology and behaviour are all the result of a deficiency of hæmoglobin synthesis and hence of erythroblast cytoplasm that is more severe than is ever seen in simple iron deficiency It is true too that in the minor and minima varieties the changes in the peripheral blood and in the bone marrow are similar to and difficult to distinguish from those produced by simple iron deficiency Even so the degree of punctate basophilia and target cell formation is usually greater than in iron deficiency

From the morphological point of view there are therefore some differences between Mediterranean anæmia and iron deficiency anæmia which do not seem to be entirely explained on quantitative differences in the severity of the impairment of hæmoglobin synthesis There are moreover some other differences which are probably of pathogenetic significance For instance patients may be encountered in whom jaundice of apparently hæmolytic type is a marked feature (the Rietti Greppi Micheli anæmia) Whether or not the unusual tendency to hæmolysis and jaundice is the result of other genetic influences in addition to that produced by the gene for Mediterranean anæmia is unknown at present

Further morphological evidence of abnormal erythropoiesis was provided by Astaldi Rondanelli Bernardelli and Strosselli (1954) who demonstrated by means of the periodic acid Schiff (PAS) reaction an abnormal substance (glucomucoprotein or mucopolysaccharide) in the cytoplasm of erythroblasts in 20 cases of thalassæmia major Either pink staining granules or less often a diffuse pink colouration was observed No reaction was discernible with normal bone marrow cells nor with the marrow of ten cases of thalassæmia minor A positive reaction was however obtained in a single patient with *leukæmia* This work has been confirmed by Chatterjea and co workers (1956) and by Greig and Metz (1957) its significance is unknown Greig and Metz however reported positive results in thalassæmia minor as well as in thalassæmia major and also weak positive reactions in two patients with hereditary spherocytosis and one with paroxysmal nocturnal hæmoglobinuria Strongly positive results were also obtained in two patients suffering from erythræmic myelosis

Another approach has been by way of electron microscopy *Hoffman* Wolman Hillier and Parpart (1956) studied the ultrastructure of erythrocyte ghosts in this way and found that although the surface texture of thalassæmia minor cells seemed to be normal that of thalassæmia major cells was abnormally coarse Bessis and Breton Gorius (1957) demonstrated in the erythrocytes in Cooley s anæmia also by means of the electron microscope a gross excess of minute dense iron containing granules scattered throughout the cytoplasm or in masses The masses of granules correspond with *the siderotic* granules visible by ordinary microscopy Their remarkably fine

50% of the patients and the remainder have from 1–4% Fessas's (1959a) figures are similar there is usually less than 3% of Hb F and often a normal content six out of 50 patients had Hb F contents of 3–7% Some data illustrating how the Hb F content of blood may be calculated are shown in Fig 93

The persistence of Hb F beyond the neonatal period in the erythrocytes in Mediterranean anæmia and in sickle cell disease

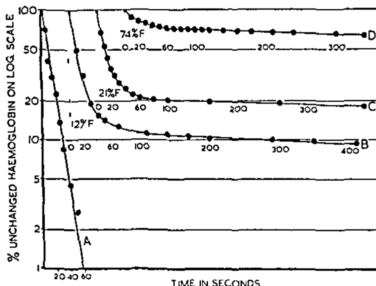


FIG 93 Denaturation rates of hæmoglobin by alkali

A Hb S trait No Hb-F present (a normal result)
B Mediterranean anæmia (thalassæmia major) 12 % of Hb F
C Hb S disease (child aged 1 year) 21 % of Hb-F
D Cord blood from a normal infant 74 % of Hb F (From White and Beaven 1954)

is of great interest The reason for its persistence is however obscure. Rich (1952) made the interesting suggestion that it was the block to the synthesis of normal adult hæmoglobin which led to the persistence of the fœtal type rather than that the gene influenced Hb F formation in a positive way This idea seems generally to have been accepted

Recently a second normal type of adult hæmoglobin (A_2) has been recognized (Kunkel and Wallenius 1955) Hb A_2 is normally present only in small quantities its mobility on paper

similarity between Hb F and Mediterranean anæmia alkali resistant hæmoglobin have been established (Liquori 1951 Singer Chernoff and Singer 1951 Rich, 1952 Chernoff 1953, Roche *et al* 1953 Itano 1957, White and Beaven 1959)

It seems likely that the alkali resistant hæmoglobin in Mediterranean anæmia erythrocytes is in fact identical with Hb F Liquori (1951) in addition to observing the increased resistance to alkali denaturation also found that the crystal form of the hæmoglobin of one patient resembled that of human Hb F as described by Jope and O Brien (1949) rather than that of human adult hæmoglobin Jope (1949) demonstrated a significant difference in the form and position of the tryptophane notch in the ultraviolet absorption spectrum of Hb F as compared with the normal adult type In thalassæmia major exactly the same difference is discernible (Liquori 1951 Beaven and White 1953)

Chernoff (1953) showed that there was a very close relationship between the amounts of foetal type hæmoglobin in various types of pathological erythrocytes as estimated by alkali denaturation and the amounts as estimated by an immunological method using a serum prepared against Hb F obtained from cord blood

Huisman Prins and van der Schaaf (1956) found that the amino acid composition solubility and behaviour on chromatography of the alkali resistant hæmoglobin of Cooley s anæmia and normal Hb F were the same and de Marco and Trasarti (1957) obtained identical data on crystallization and for amino acid composition

There is thus an impressive array of evidence in favour of the absolute identity of the alkali resistant hæmoglobin in thalassæmia and Hb F Nevertheless it has to be added that Dernen (1959) states that if the two hæmoglobins are subjected to moving boundary electrophoresis at pH 6 5 and ionic strength 0 018, totally different diagrams are obtained

Many quantitative measurements of alkali resistant hæmoglobin in thalassæmia have by now been carried out In thalassæmia major Hb F is usually present in very large amounts and values of 50–90% are usual (Cutillo and Romagnoli 1953 Penati Turco and Lovisetto 1954) However percentages as low as 20% or even less have been observed in patients who appear to be genuinely homozygous for the thalassæmia gene (Choremis Zannos and Dentakis 1955 Sturgeon Itano and Bergren 1955 White and Beaven 1959) and it seems generally to be agreed that the proportion of Hb F present cannot be correlated with the clinical severity of the disease (*e g* Penati *et al* 1955 Sturgeon Itano and Bergren 1955 Fessas 1959a)

In thalassæmia minor the amounts of Hb F present are always small According to Beaven and White (1959) who have used several sensitive methods of estimation none is detectable in about

content exceeded 4 5% 7% were normal or borderline The results in thalassæmia major were variable ranging from subnormal levels to 13 3 / There appeared to be no correlation between the hæmoglobin level and the Hb A_2 content

SYNDROMES PROBABLY ALLIED TO MEDITERRANEAN ANÆMIA

1 Sex linked Anæmia of Cooley and Rundles and Falls (Pseudothalassæmia)

In recent years a small number of family studies have been reported of a hereditary hypochromic anæmia which appears to be distinct from Mediterranean anæmia Subjects of varying nationalities have been affected As in Mediterranean anæmia the essential defect seems to be a failure of the synthesis of hæmoglobin whether or to what degree excess hæmolysis is important is uncertain It seems likely that the majority of these reports deal with the same disorder which has been variously named (see references) Pseudothalassæmia (Gelpi and Ende 1958) is the only short title which has been suggested The disorder is remarkable in appearing to affect in a severe form only males The first descriptions available are those of Cooley (1945) and Rundles and Falls (1946) and as an alternative title to pseudothalassæmia the eponymous one Sex linked anæmia of Cooley and Rundles and Falls might be appropriate but for the fact that it is too long and associates Cooley with *two* superficially similar but apparently distinct types of hereditary anæmia The title Anæmia hypochromica sideroachrestica hereditaria of Heilmeyer and co workers (1958) while appropriate also suffers by being too long and is too stiff Malassenet (1958) in a recent review refers to anémie hypochrome hypersidérémique This title however fails to indicate the hereditary nature of the disorder

As already mentioned the first description was that of Cooley (1945) who under the title A severe type of hereditary anemia with elliptocytosis reported the incidence of an unusual type of anæmia in two brothers They were members of a family in which for five generations back on the mother s side 19 out of 29 males had suffered from severe anæmia Sixteen of them had died ten in their first year Neither the boys mother nor any other female relative was affected The boys erythrocytes were markedly hypochromic and more than 50 / of the cells were oval or elliptical in shape No target cells were present Osmotic fragility studies showed an increased span of resistance there were a few fragile cells but on the whole resistance was increased One boy underwent splenectomy but without definite improvement

electrophoresis is similar to that of Hb E and it is best demonstrated by starch block electrophoresis Kunkel and Wallenius found increased levels of the new component in thalassæmia minor—its presence was probably the cause of the abnormal electrophoretic pattern which had been noted by Humble Anderson and White (1954) and Singer and co workers (1954) in single cases More recent studies have confirmed these observations (Marinone *et al* 1956 Kunkel *et al* 1957 Crowley *et al* 1957 Carcassi Ceppellini and Siniscalco 1957 Marinone and Bernasconi 1958 Gerald and Diamond 1958a Josephson *et al* 1958 Fessas 1959a)

Kunkel and co workers (1957) found the A_2 component to be present in the blood of each of 300 adults (mean concentration 2 54 ± 0 35% of the total hæmoglobin present) In thalassæmia minor higher values were obtained (mean 5 11 ± 1 36%) They made the point that in its virtual absence at birth the findings with Hb A_2 parallel those with Hb S Hb C and Hb E Normal values for Hb A_2 were found in the presence of single or double genes for Hb S suggesting that the gene for Hb A_2 is at a different locus The concentration of Hb A_2 was found to be low in thalassæmia major Kunkel and his colleagues suggested that the rise in the Hb A_2 level in thalassæmia minor is equivalent to the rise in Hb C or Hb S when heterozygosity for Hb C or Hb S is combined with the presence of a single gene for thalassæmia

Carcassi Ceppellini and Siniscalco (1957) compared the Hb A_2 content of the blood of 78 patients with thalassæmia minima with that of 157 normal subjects The mean values were 5 19 ± 0 68% and 2 33 ± 0 39% respectively In a few patients a raised Hb A content was the only abnormality detected In contrast there were also a few undoubted carriers of the thalassæmia gene whose Hb A_2 contents were normal some of the latter group were iron deficient

Gerald and Diamond (1958a) studied six thalassæmic families As expected the parents of children with thalassæmia major all showed an increase in the concentration of the A_2 component as well as microcytosis However there appeared to be differences in the concentration of Hb A_2 between different families although the concentrations within families were similar Gerald and Diamond stressed the value of the measurement of Hb A_2 and mean corpuscular volume in the diagnosis of thalassæmia trait

The data of Josephson and his co workers (1958) were essentially similar Thirty two patients with thalassæmia intermedia minor or minima were studied in each case the Hb A_2 content exceeded 3 5% the upper limit of normal Two children with Cooley s anæmia were also investigated and contrary to the results of Kunkel and his co workers (1957) abnormally high values for Hb A_2 (4 3% and 9 4%) were found Josephson and his colleagues failed to find any correlation between the severity of the disease and the content of Hb A_2

Fessas (1959a) reported on 51 patients with thalassæmia minor and twenty with thalassæmia major in 90% of the minor cases the Hb A_2

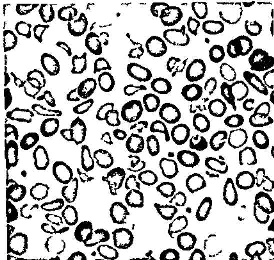


FIG 94 Photomicrograph of a blood film of a young m n believe l to be uffering from th e sex l nked anæmia of Cooley and Rundle and I all (pseudotl alassæmia) Before splenectomy Tl e blood film i similar to that of an intermed ate grade of Mediterranean anæmia Con iderable anisochroma ia i present but no derocytes × 700

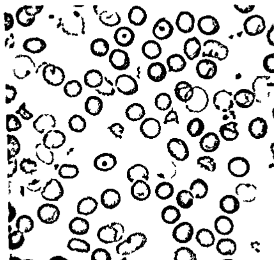


FIG 9 Pl otom ograph of a blood film of a young man bel eved to be suffering f om the sex l nked anæmia of Cooley and Rundles and I all (ps udotl ala sæm a) Afte spl nectomy and aft r tr n fus on Numerou siderocyte are pre ent The da kly stain d cell a e tr sfu d cell Note th t se eral of tl em have a su ed a tar t form × 700

Rundles and Falls (1946) described two further American families probably suffering from the same disorder The first family was of German Scottish and English origin in this family there were two relatively severely affected males and five mildly affected females The second family was of English Dutch and Swiss stock two boys were severely affected and there were six mildly affected females The most obvious blood abnormalities in the mild (carrier condition) were anisocytosis and the presence of some hypochromic elliptocytes and poikilocytes In the severely anæmic males the intensity of the variation in erythrocyte size and shape and staining closely simulated that found in Mediterranean anæmia of a comparable degree of anæmia One of the patients underwent splenectomy without benefit Inclusion bodies (siderotic granules) were noticed in his erythrocytes after the operation

More recent reports describing patients who probably have suffered from the same or a closely related disorder are summarized below All the descriptions refer to males

Mills and Lucia (1949) described the occurrence in a man aged 27 of German Irish and French descent of a refractory hypochromic microcytic anæmia Numerous siderocytes were noticed in his erythrocytes after splenectomy His anæmia was considered to be distinct from thalassæmia it was attributed to a defect in the ability of the hæmopoietic system to utilize iron The patient s half brother had an identical condition and coarse siderotic granules were noted in his normoblasts He also underwent splenectomy Both brothers ultimately died of anæmia cardiac failure and multiple thromboses

More recently Garby Sjolin and Vahlquist (1957) have described detailed studies on a boy aged 15 years suffering from a chronic refractory hypochromic anæmia Other members of his family were probably also affected Thalassæmia was excluded as the diagnosis on the grounds that the spleen was not palpable despite severe anæmia The fœtal hæmoglobin concentration was very low (0 7%) and the life span of the patient s erythrocytes was normal Disturbed hæm metabolism was suggested as the probable cause of the anæmia

Lukl Wiedermann and Barborik (1958) described the incidence of a thalassæmia like condition in five adult male patients (of Czech origin) which was associated with hæmochromatosis demonstrated by liver biopsy or at necropsy The hæmatological findings were considered to be almost identical with those of thalassæmia and in support of this view the authors instanced the leptocytosis (but no erythroblastosis) the increased erythrocyte osmotic resistance the hypersiderinæmia and what they considered to be identical bone marrow changes including positive Hotchkiss (PAS) reactions Anæmia was severe with the hæmoglobin concentration in one patient falling as low as 3 2 g per 100 ml Abnormal hæmoglobins were not demonstrated and the Hb F content was only slightly raised (3 8–6 9%) The MCV varied from 62 to 89 cu μ MCHC from 22 to 27% and MCD from 6 9 to 7 3 μ The highest reticulocyte count recorded was 6% Two of the patients died and the third was being kept alive by blood transfusions An extensive family study revealed that the patients were probably not homozygotes for an abnormal gene and that the disorder was severe only in males but could be transmitted by females who might be mildly affected (exactly as in the family study of Rundles and Falls (1946)

Heilmeyer and his co-workers (1958) reported similar studies on a 34 year old man and his brother also suffering from a hypochromic anæmia refractory to treatment Siderotic granules in the erythroblasts were unusually large and numerous The absence of evidence of increased hæmolysis absence of target cells and the normal mean cell diameter were thought to differentiate the disorder from thalassæmia However neither patient was particularly anæmic (hæmoglobins 10 4 and 10 8 g per 100 ml respectively)

Gelpi and Ende (1958) described two brothers of Swiss English stock aged 33 and 39 years respectively Their mother and a sister may have had the disorder in a mild form The more severely affected of the patients was submitted to splenectomy Benefit was short lived and subsequently he suffered from recurrent phlebitis The platelet count ranged between 2 to 3 millions per cu mm There was some evidence of excess hæmolysis the ^{51}Cr half time of the patient s cells was 19½ days (that of normal cell in the patient was 35 days) The life span of the brother s erythrocytes was normal (he was less anæmic hæmoglobin 10 g per 100 ml) Serial liver biopsies in the more severely affected patient demonstrated the progressive development of hæmochromatosis and ultimately glucose tolerance tests gave diabetic type curves

As mentioned in the first edition of this book (Dacie 1954 p 130) the author has made some observations on a family living in London who almost certainly suffer from the same type of sex linked anæmia as is described in the reports mentioned above Pre splenectomy and post splenectomy blood films of the most severely affected patient (a young man) and of his mother are illustrated in Figs 94–96 Since then another patient (also a young man) has been studied who presented with an identical blood picture (after splenectomy)

In many respects the above disorder simulates Mediterranean anæmia However there are some differences of which the most important is clearly the mode of inheritance (the gene being dominant but only achieving full expression in males) If this mode of inheritance can be confirmed in other families the separation of sex linked anæmia from Mediterranean anæmia can be considered as proved The blood pictures of the two conditions are similar if not identical However it is possible that the number and size of siderotic granules in developing erythroblasts may prove to be greater in sex linked anæmia than in Mediterranean anæmia (*cf* Figs 97 and 97) Further study on this point is required Another possibly distinguishing point is the relatively low or even normal levels of Hb F in sex linked anæmia (It can however be argued that low levels of Hb F may also be found in clinically severe thalassæmia minor with comparable degrees of anæmia)

Not only is the marrow iron greatly increased in sex linked anæmia but there is also a general tendency to the deposition of iron throughout the body particularly in the liver It does not

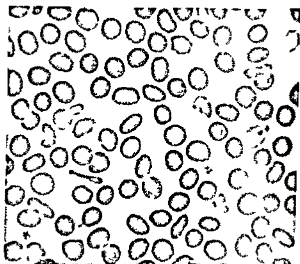


Fig 96 Photomicrograph of a blood film of the mother of the young man whose films are illustrated in Figs 94 and 9. She had a mild hypochromic anæmia There is a moderate degree of anisocytosis anisochromasia and poikilocytosis × 700



Fig 97 Photomicrograph of the bone marrow film of the young man whose blood films are illustrated in Figs 94 and 95 Three normoblasts are shown in the cytoplasm of which is a massive accumulation of iron containing granules Perls's acid ferrocyanide reaction × 1100

present in heterozygous form with normal hæmoglobin has an effect on erythropoiesis which is closer to that of thalassæmia than the other hæmoglobinopathies

3 *Other as yet Unclassified Syndromes*

As described on p 267 there have been several reports which suggest that an abnormal gene exists whose effect is to permit the persistence into adult life of relatively large amounts of fœtal hæmoglobin Its presence is however not associated with anæmia or any other recognizable hæmatological abnormality This disorder is thus easily separable from Mediterranean anæmia

Cabannes Raffi and Boineau (1957) have reported another interesting abnormality the presence in several members of a family suffering from a congenital hæmolytic anæmia of excessive amounts of Hb A_2 (14–27 %) amounts far higher than are usually found in thalassæmia minor Cabannes and his colleagues suggested that the abnormality in this family also belongs to the thalassæmia group rather than to the group of abnormal hæmoglobinopathies

Other patients whose blood pictures have shown unusual features *e g* macrocytosis and a high colour index have been described by Gasser (1951) and Schaeffer (1954) The exact status of these cases (thalassæmia of the Fanconi Patrassi type) is obscure The same is true of the two children described by Debler (1939–40) as suffering from a familial hypochromic hæmolytic anæmia with increased osmotic fragility and who responded well to splenectomy Perhaps this disorder belongs to the group referred to by Quattrin (1950) as an intermediate type of constitutional hæmolytic jaundice due he believed to the intermarriage between carriers of the traits for hereditary spherocytosis Rietti Greppi Micheli anæmia or hereditary elliptocytosis

The familial congenital chronic hæmolytic anæmia of Stransky and Regala (1940) is now known to belong to the Hb E syndromes

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seem likely that this can be wholly accounted for by iron given in the form of transfused blood It is uncertain whether the excess iron is derived from the increased absorption of therapeutically administered iron or of iron in foodstuffs as the result simply of a state of anæmia persisting for years or whether there is an increased avidity for absorbing iron unconnected with anæmia It is clear however that (hæmochromatosis figures prominently in reports of sex linked anæmia (e g those of Lukl Wiedermann and Barborík (1958) and Gelpi and Ende (1958))

Treatment Splenectomy is inadvisable The anæmia is not significantly affected and post operative thrombophlebitis is apparently a frequent and serious complication *e g* in the two patients of Mills and Lucia (1949) and the patient of Gelpi and Ende (1958) Thrombophlebitis and pulmonary embolism also led to the death of one of the patients studied by the author

Pyridoxine has been used repeatedly in refractory anæmia of obscure origin and although most patients do not respond there are reports of at least two patients (who may or may not have had sex linked anæmia) who did respond (Harris *et al* 1956 Bishop and Bethell 1958 see also Dacie *et al* (1959)) Pyridoxine should thus be tried but any benefit that results seems likely to be due to relief of a secondary deficiency such as might be produced by marked siderosis rather than due to alleviation of the fundamental disturbance in hæmoglobin synthesis

2 *The Lepore Trait*

Gerald and Diamond (1958b) have recently described an infant of Italian parents who was thought to be homozygous for thalassæmia but who on further investigation was shown to be a double heterozygote for thalassæmia (inherited from his father) and Lepore hæmoglobin (inherited from his mother) (Lepore hæmoglobin can apparently only be clearly separated from Hb A by starch block electrophoresis it then migrates between Hb A and Hb A_2 at the same speed as Hb S It does not cause sickling)

Lepore hæmoglobin was demonstrated in several members of the mother s family (10–12% of abnormal hæmoglobin) Its presence was associated with a blood picture resembling that of thalassæmia minor there was little or no anæmia but some target cells could be seen in films and there was a moderate degree of anisocytosis and poikilocytosis The MCV was between 71 and 76 cu μ the MCHC was normal (32–34%) The infant propositus (heterozygous for thalassæmia and the Lepore trait) was severely anæmic (hæmoglobin 5 7 g per 100 ml) its blood film resembled that of thalassæmia major 74 % of its hæmoglobin was Hb F [illegible]% was Lepore hæmoglobin and 1 2% Hb A_2

Gerald and Diamond pointed out that Lepore hæmoglobin by giving rise to a clinical syndrome with mild anæmia and microcytosis when

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CHAPTER 6

THE HEREDITARY HÆMOGLOBINOPATHIES

SICKLE-CELL DISEASE AND ALLIED SYNDROMES

History In 1910 Herrick published an article entitled Peculiar elongated and sickle shaped red blood corpuscles in a case of severe anemia in which were described many of the more characteristic hæmatological and clinical findings of what was later referred to as sickle cell anæmia Although sickle cells were well illustrated by Herrick the development of sickling *in vitro* was not described until 1915 when Emmel studying the blood of a patient whose clinical history was reported by Cook and Meyer (1915) noticed that long sharp projections formed from the erythrocytes when sealed preparations of blood were allowed to stand undisturbed at room temperature for several days In 1917 Emmel published a full description of his observations on the development of sickled forms he also reported that identical changes took place when the blood of the patient s father was similarly cultured *in vitro* Mason (1922) introduced the term sickle cell anemia and suggested that the disease might be confined to the negro race In 1923 Huck showed that the sickling phenomenon was unquestionably inherited and an analysis of his data by Taliaferro and Huck (1923) indicated that the inheritance was probably controlled by a single non sex linked abnormal gene acting as a Mendelian dominant Out of 23 children in a single family born of matings between presumed heterozygotes and normal subjects eleven were normal eleven showed sickling and one was untested

Full clinical accounts soon followed Sydenstricker s (1924) paper is particularly noteworthy active and latent phases of the disease were recognized and the anæmia was attributed to excessive blood destruction secondary to the sickling phenomenon The blood changes were described in detail The previous year Sydenstricker Mulherin and Houseal (1923) had described the post mortem findings in two cases They also tested the blood of a large number of white and negro subjects for sickling and

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The literature on the abnormal hæmoglobins is now vast in extent In 1951 Margolies listed 344 references in a comprehensive review on sickle cell anæmia Since then however many hundreds more papers have been published Important reviews covering the clinical genetical and chemical aspects of this new knowledge include those of Pauling (1953–54) Itano (1955 1956 1957) White and Beaven (1954) Singer (1951 1955) Chernoff (1955) Allison (1956b) Lehmann (1956 1957 1959a) Zuelzer Neel and Robinson (1956) Watson (1956) Itano Bergren and Sturgeon (1956) Gatto (1956) Smith (1957) Neel (1957) Huisman (1958) Ingram (1959) and Beaven and Gratzer (1959)

The alphabetical nomenclature of the recently discovered hæmoglobins has fortunately become standardized and generally accepted and in this Chapter I shall adhere to the suggestions contained in a comparatively recent publication (1957) of the British Colonial Medical Research Committee They describe a hæmoglobinopathy as a condition in which the production of normal adult hæmoglobin (Hb A) is partly or wholly suppressed and is partly or wholly replaced by one or more hæmoglobin variants which may include fœtal type hæmoglobin (Hb F) The term disease is used to refer to any morbid condition produced by one or two hæmoglobin variants in the absence of Hb A Combinations of one abnormal hæmoglobin with Hb A give rise to traits which with the possible exception of sickle cell trait (Hb S trait) have no clinical effects (see p 251)

The short notation recommended by a Working Party of the Colonial Medical Research Committee is as follows for an abnormal hæmoglobin (*e g* Hb C) in homozygous state Hb C disease for the combination of two abnormal hæmoglobins (*e g* Hb C plus Hb D) Hb C/Hb D disease (hypothetical) or of one hæmoglobin (*e g* Hb C) and thalassæmia Hb C/thalassæmia Combinations of an abnormal hæmoglobin (*e g* Hb C) with normal hæmoglobin are written simply as Hb C trait etc

The Committee appears to prefer the term sickle cell trait for Hb S trait although using the notation Hb C trait and Hb D trait etc In the present work however I shall use Hb S as a convenient short notation for hæmoglobin S and also Hb S trait Hb S disease and Hb S/Hb C disease for sickle cell trait sickle cell disease and sickle cell/Hb C disease respectively

In this Chapter the clinical syndromes associated with the presence of the various abnormal hæmoglobins will be considered first Next more details will be given of the abnormal hæmoglobins and finally the pathogenesis and treatment of the clinical

concluded that sickle cell anæmia was a familial disease which only affected the negro race

Subsequent discoveries of great significance include those of Hahn and Gillespie (1927) who showed that sickling developed as the result of a fall in the partial pressure of oxygen and the more recent work of Pauling Itano Singer and Wells (1949) who demonstrated that the sickling phenomenon was associated with the presence of an abnormal form of hæmoglobin (hæmoglobin S or Hb S) The work of Pauling and his collaborators has been the starting point of much recent work of major importance In particular at least 14 further types of abnormal adult hæmoglobin in addition to sickle cell hæmoglobin have been discovered and a whole range of new clinical syndromes have been recognized

The Abnormal Hæmoglobins The new' hæmoglobins have been named alphabetically in order of discovery However hæmoglobin S (Hb S) is used to describe sickle cell hæmoglobin rather than hæmoglobin B and hæmoglobin F (Hb F) is retained for fœtal hæmoglobin Normal adult hæmoglobin is referred to as hæmoglobin A (Hb A)

Hæmoglobin C (Hb C) was described by Itano and Neel in 1950 and hæmoglobin D (Hb D) by Itano in 1951 Hæmoglobin E (Hb E) was described independently in 1954 by Chernoff Minnich and Chongchareonsuk and by Itano Bergren and Sturgeon and hæmoglobin G (Hb G) by Edington and Lehmann (1954b) Since 1954 hæmoglobins H I J K L N O P and Q have been discovered and the list does not yet seem to be complete (see p 290)

Unfortunately the convenient alphabetical system of nomenclature seems at present to be breaking down First it is possible that all the available letters may soon be used up! Secondly it has happened that the same letter has been allotted to more than one type of hæmoglobin as the result of new discoveries being made more or less simultaneously in different parts of the world To avoid this and to prevent a hæmoglobin being labelled as a new one when in reality it is the same as another recently described variety several workers have tentatively labelled their new hæmoglobins according to where the hæmoglobin was found e g Galveston (Schneider and Haggard 1957) Barts (Ager and Lehmann 1958) and Norfolk (Ager Lehmann and Vella 1958) Ager Lehmann and Vella (1958) have suggested that it may be useful as a temporary measure to separate the abnormal hæmoglobins into several main groups according to their general type of electrophoretic mobility and to label the hæmoglobins within each group numerically or by small letters e g Group I or C (those types which migrate the slowest at pH 8 6) Group II or S Group III or G Group IV or A, Group V or J and Group VI or I (the group which migrate the fastest)

(1951) listed thirteen instances mostly in Greeks Italians and Sicilians but added that ancestral negro blood can be suspected Margolies (1951) who referred to 30 cases and Plachta and Speer (1952) similarly concluded that ancestral admixture with negro blood was the most likely explanation

Present knowledge is summarized in Fig 98 Hb S is found all over tropical Africa although not equally distributed amongst the African races—the maximum incidence of the Hb S trait is about 40% It is also found to some extent in limited loci in the countries bordering on the Mediterranean in the Middle East and in India (see Lehmann 1956 1956–57 1959a Neel 1957)

The relative incidence of the Hb S trait in different populations was comprehensively reviewed by Mourant (1954) More recent reviews on the distribution of Hb S in Africa include those of Allison (1956c) Neel (1957) and Vandepitte (1959)

Lehmann and Cutbush (1952) were the first to find Hb S in India (in certain primitive hill tribes (Veddoids)) Subsequently Hb S trait and Hb S disease were identified in other places in India and Assam (see Shukla and Solanki 1958 Shukla Solanki and Parande 1958 Chatterjea 1959) Choremis and co-workers (1951) found that the Hb S trait was relatively frequent in certain localities in Greece (see also Fessas 1959b) and in Turkey Aksoy (1955 1956) described a relatively high incidence of Hb S trait (13 3%) and also Hb S disease in an isolated Eti Turk community (see also Aksoy 1959) Cabannes and co workers (1956) and Portier Cabannes and Duzer (1959) have provided data on the occurrence of Hb-S (and other abnormal hæmoglobins) in North Africa

Whether the gene responsible for the Hb S trait has risen independently in the different ethnic groups referred to above or has spread to each group from a common ancestor is an unsettled and intriguing problem (Neel 1953) Lehmann (1953) suggested that the Hb S trait was not an essentially negroid feature he considered that the trait had probably entered Africa from the north east According to Lehmann (1956–57 1959b) Veddoid races peopled Arabia in neolithic times spreading south into Africa and India later perhaps carrying the gene for Hb S with them (Fig 99)

The presence of Hb S in America is by contrast a relatively recent event it was undoubtedly brought to the West Indies (Jelliffe Stuart and Wills 1954) and to North America first in the 17th century by the importation of negro slaves from West Africa The present incidence in the negro population in the United States is 8–9% or almost 1% of the entire population (Schneider 1956 Levin 1958)

Inheritance of Hæmoglobin S The possibility that the sickle cell phenomenon might be inherited was first hinted at by Emmel (1917) who observed that the blood of the father of a patient suffering from sickle cell anæmia sickled *in vitro* In 1923 as has already been mentioned Huck and Taliaferro showed that

syndromes will be discussed Sickle cell anæmia (Hb S disease) receives the fullest treatment it is the most serious of all the hæmoglobinopathies and has been the most fully studied

SICKLE CELL TRAIT AND SICKLE CELL DISEASE

Synonyms *Sickle cell trait* Hb S trait sicklemia (Cooley and Lee 1926) sicklemia trait (Committee for Clarification of Nomenclature, 1950) *Sickle cell disease* sickle cell anemia (Mason, 1922) drepanocytic anemia (Hahn 1928) meniscocytic anemia



FIG 98 The distribution of Hb S in the Old World (Only areas where sickling has been found repeatedly have been marked with one exception—the remarkable observation of sickling in Bihar coolies working in Assam) Redrawn from Lehmann (19 9b)

(Graham and McCarty 1930) sicklemia (Committee for Clarification of Nomenclature 1950)

Geographical Distribution of Hæmoglobin S Much work has been done in recent years on the incidence and geographical distribution of Hb S

As already mentioned the early work in America suggested that the Hb S trait was entirely confined to the blood of negroes Later, however doubts arose and Mason (1938) accepted as authentic reports of sickling in white families without any reasonable suspicion of admixture with negro blood Wintrobe

time to time for no apparent reason in a drepanocytic population Valuable large scale family studies carried out in the Belgian Congo by the Lambotte Legrands (1950–51 1952 1955a) and by Vandepitte (1954) provided further valuable evidence in support of the hypothesis of Neel and Beet As will be described later the proportions of Hb S as determined by electrophoresis in Hb S trait and Hb S disease are also strongly in favour of the hypothesis that the trait represents the heterozygous state and the disease the homozygous state of an abnormal gene

The gene for Hb S is generally considered to be an allele of normal adult hæmoglobin (Hb A) Hb C and Hb D are probably further alleles at the same locus Hæmoglobin G and the gene for thalassæmia are apparently not alleles of Hb A or of each other The genes controlling hæmoglobin formation thus occupy at least three loci (Schwartz *et al* 1957 Neel 1957 1958 1959 Zuelzer 1957) No linkages between the gene for Hb S and the genes responsible for the blood groups or other easily recognizable inherited characters have been established (Neel Schull and Shapiro 1952)

As will be described later sickle cell disease (sickle cell anæmia) is a serious disorder with a high mortality in infancy and children How is it therefore that the gene for Hb S is so widespread if the homozygotes so often die before reaching maturity? This has been the subject of much debate and speculation and it now seems to be generally agreed that the high incidence of the Hb S gene in some parts of the world is brought about by a state of balanced polymorphism with the heterozygote condition having a positive survival value over the normal and abnormal homozygotes The alternative possibility that the Hb S gene could be maintained by fresh mutations appears to be highly improbable it would imply a rate of mutation many times greater than other known mutation rates (Allison 1954a b c Vandepitte 1954 J and C Lambotte Legrand 1955 Vandepitte Zuelzer Neel and Colaert 1955 Neel 1957 1959)

As will be discussed in the following section it is now widely agreed that a relative resistance to *P falciparum* malaria has been a most important mechanism in bringing about the persistence and high frequency of the Hb S gene

Resistance to Malaria

Brain (1952) seems to be the first to have suggested in print that the erythrocytes of sicklers might offer a less favourable environment for malaria parasites than normal cells He based this suggestion on the observation of Beet (1947) confirmed by himself that the spleen which he (Brain) presumed was enlarged due to malarial infection was less frequently palpable in sicklers than in non sicklers

sickling was inherited as a non sex linked Mendelian dominant It was realized by Sydenstricker (1924) that latent and active forms of sickle cell anæmia occurred and that latent cases far outnumbered active cases Later the two forms became generally known as sickle cell anæmia and (symptomless) ' sickle cell trait' (Cooley and Lee 1926 Diggs Ahmann and Bibb 1933–34 *Sherman 1940*)

The exact relationship between the anæmia and trait was not established until considerably later In 1947 Neel stated This



FIG 99 The *origin of the Hb S gene An illustration of the theory* suggesting that the gene for sickling arose in the Middle East amongst the Veddoids and spread from there into Africa and into India Redrawn from Lehmann (1959b)

[modification of Huck s hypothesis] is that there is present in the coloured population a certain factor which when heterozygous may have no discernible effects but usually results in sickling and when homozygous tends to result in sickle cell anemia Proof of this hypothesis was published by Neel (1949) when he reported that the erythrocytes of all of 42 parents of 29 patients with sickle cell anæmia sickled Further confirmatory data were published by Neel in *1951 Beet (1949) independently came to the same* conclusion as did Neel based on the study of a single large family he stated that sickle cell anemia occurs in homozygotes only and that this may account for the occurrence of the anæmia from

of Hb S and the parasite counts remain low The clinical effect of this is that the infection is of short duration the incidence of cerebral malaria is low and the mortality is also low Allison concluded by stating that if the child mortality in Africa due to malaria was 10% and if this was confined solely to non sicklers then this could explain the high incidence of Hb S in Africa

It must be added that while the importance of malaria is generally accepted the possibility remains that other factors also aid in the survival of the Hb S gene (Foy *et al* 1955 Neel 1957 J and C Lambotte Legrand 1958) Delbrouck (1958) even concluded that in the Belgian Congo hyperfecundity was more important in this respect than resistance to malaria

Clinical Features of the Sickle Cell Trait (Hb S Trait)

Until recently it has generally been assumed that the presence of the Hb S trait is not associated with any clinical effects whatsoever This is undoubtedly true in the great majority of instances Nevertheless symptoms and pathological changes occasionally occur which seem most easily explained by the presence of the trait these symptoms are hæmaturia splenic infarction during aeroplane flights and less certainly cerebral thrombosis and pulmonary infarction (see Singer 1955 Levin 1958)

Hæmaturia Judging from recent reports hæmaturia is a not very infrequent complication of the Hb S trait In 1950 Goodwin Alston and Semans described a series of negro patients whose blood sickled and who had developed unexplained hæmaturia Four of the patients were considered to have the Hb S trait Further similar cases were reported by Lund Cordonnier and Forbes (1954) and Chapman and co workers (1954) Goldman Chapman and Cross (1955) investigated six patients with Hb S trait who had had hæmaturia on several or innumerable occasions No urological cause could be found and in six of the patients it was noted that at the time of investigation the bleeding was confined to one kidney Mostofi Vorder Bruegge and Diggs (1957) studied the pathology of the kidney removed from 22 young negro patients thought to have Hb S trait Severe congestion with focal medullary and papillary hæmorrhages and areas of necrosis repair and regeneration were the lesions demonstrated In a review of the literature they referred to 58 patients they stressed that the bleeding may be unilateral and that nephrectomy should be avoided Further *patients with electrophoretically proven Hb S trait and gross* hæmaturia were reported by Crone and co workers (1957)

Beet (1946) had previously reported that the incidence of malaria parasites was lower in sicklers than in non sicklers in the Balovale district of Northern Rhodesia However the difference in incidence between the two groups was found to be much less obvious when a similar study was carried out in another part of the country and the possible importance of his first observation was not appreciated (Beet 1947) Unfortunately too Beet interpreted the finding of a reduced incidence of splenomegaly in sicklers as due to infarction of the spleen rather than to a diminished incidence of malaria

At about the same time as the above mentioned work was being carried out in East Africa a relationship between the presence of sickling and malaria was also beginning to be appreciated in the Belgian Congo J and C Lambotte Legrand (1950–51) reported that the general mortality was lower in infants who were sicklers than in non sicklers and that, although the incidence of a variety of infections was about the same in the two groups the mortality from malaria and the incidence of cerebral attacks were much lower in sicklers than in non sicklers

It was left to Allison (1954a b c) to provide the first definite evidence that the hypothesis that a person who was heterozygous for Hb-S might have an increased resistance to malaria was likely to be correct and that the advantage applied apparently only to infection with *P falciparum* (subtertian malaria) The importance of malaria in ensuring the persistence of the gene for Hb S seems now to be generally accepted (Edington and Lehmann 1956 Lehmann and Raper 1956 Raper 1955 1956 Allison 1957a Vandepitte and Delaisse 1957 J and C Lambotte Legrand 1958 Neel 1959 Lehmann 1959a b c)

Hb S trait occurs at its highest frequency in areas where malaria is hyperendemic this applies to its incidence in Greece and India as well as in Africa In North America the incidence of Hb S trait (about 9%) is less than that of the trait in the areas of Africa from which the negro slaves were imported (15 4% Allison 1954b) This fall in incidence has according to Allison been brought about by the removal of the sicklers from a malarial environment In Africa in areas where malaria is hyperendemic the parasite counts in children who are sicklers are lower than in non sicklers (Allison 1954a Raper 1955 Vandepitte and Delaisse 1957) Similarly serious complications such as cerebral malaria blackwater fever or severe anæmia are much less frequently met with in sicklers than non sicklers (J and C Lambotte Legrand 1952 1958 Raper 1956) It thus seems that the presence of Hb S although it does not prevent malaria limits the severity of *P falciparum* infection

It is now realized that the advantage conferred by the presence of Hb S is chiefly confined to young children before the development of immunity to malaria In adults exposed to experimental infections the difference in resistance is less clear cut (Beutler Dern and Flanagan 1955 Liachowitz *et al* 1958)

Allison (1957a) has summarized the relationship between malaria and sickling as follows Sicklers are easily infected by *P falciparum* but although the infection continues for a normal length of time the multiplication of the trophozoites is diminished in the presence

of Hb S and the parasite counts remain low. The clinical effect of this is that the infection is of short duration, the incidence of cerebral malaria is low and the mortality is also low. Allison concluded by stating that if the child mortality in Africa due to malaria was 10% and if this was confined solely to non-sicklers then this could explain the high incidence of Hb S in Africa.

It must be added that while the importance of malaria is generally accepted the possibility remains that other factors also aid in the survival of the Hb S gene (Foy *et al.* 1955; Neel, 1957; J. and C. Lambotte-Legrand, 1958). Delbrouck (1958) even concluded that in the Belgian Congo hyperfecundity was more important in this respect than resistance to malaria.

Clinical Features of the Sickle-Cell Trait (Hb S Trait)

Until recently it has generally been assumed that the presence of the Hb S trait is not associated with any clinical effects whatsoever. This is undoubtedly true in the great majority of instances. Nevertheless, symptoms and pathological changes occasionally occur which seem most easily explained by the presence of the trait; these symptoms are hæmaturia, splenic infarction during aeroplane flights and, less certainly, cerebral thrombosis and pulmonary infarction (see Singer, 1955; Lehmann, 1958).

Hæmaturia. Judging from recent reports hæmaturia is a not very infrequent complication of the Hb S trait. In 1950 Goodwin, Alston and Semans described a series of negro patients whose blood sickled and who had developed unexplained hæmaturia. Four of the patients were considered to have the Hb S trait. Further similar cases were reported by Lund, Cordonnier and Forbes (1954) and Chapman and co-workers (1954). Goldman, Chapman and Cross (1955) investigated six patients with Hb S trait who had had hæmaturia on several or innumerable occasions. No urological cause could be found and in six of the patients it was noted that at the time of investigation the bleeding was confined to one kidney. Mostofi, Vorder Bruegge and Diggs (1957) studied the pathology of the kidney removed from 22 young negro patients thought to have Hb S trait. Severe congestion with focal medullary and papillary hæmorrhages and areas of necrosis, repair and regeneration were the lesions demonstrated. In a review of the literature they referred to 58 patients; they stressed that the bleeding may be unilateral and that nephrectomy should be avoided. Further patients with electrophoretically proven Hb S trait and gross hæmaturia were reported by Crone and co-workers (1957).

Beet (1946) had previously reported that the incidence of malaria parasites was lower in sicklers than in non sicklers in the Balovale district of Northern Rhodesia However the difference in incidence between the two groups was found to be much less obvious when a similar study was carried out in another part of the country and the possible importance of his first observation was not appreciated (Beet 1947) Unfortunately too Beet interpreted the finding of a reduced incidence of splenomegaly in sicklers as due to infarction of the spleen rather than to a diminished incidence of malaria

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confusion and to the diagnosis of rare combinations such as Hb S/thalassæmia in children suffering from the Hb S trait and iron deficiency anæmia of nutritional origin

Electrophoresis of Hæmoglobin About 22–45% of the hæmoglobin in Hb S trait is Hb S (Wells and Itano 1951, Zuelzer Neel and Robinson 1956) Significant intrafamily differences in the proportion of Hb S to Hb A apparently exist (Neel Wells and Itano 1951) Neel (1952) explained this by suggesting that other genes (not necessarily those for abnormal hæmoglobins) might influence significantly the amount of Hb S formed as the result of the presence of the gene for Hb S Fœtal hæmoglobin is present in normal amounts in infants In older children and adults it is usually absent or at the most present in traces (Chernoff 1953 White and Beaven 1959) Abnormally high concentrations of Hb F are suggestive of Hb S/thalassæmia (see p 267)

Clinical Features of Sickle Cell Disease (Hb S Disease or Sickle Cell Anæmia)

Natural History and Symptoms Sickle cell disease is essentially a disease of childhood indeed the mortality is high and relatively few patients reach adult life even when the standard of medical care is high Under primitive conditions the mortality in childhood has been so high that at one time the very existence of Hb S disease as opposed to the trait outside America was doubted A reinvestigation of this apparent anomaly has shown that this view was false and wherever there have been facilities for careful studies and the Hb S trait has been present in a relatively high proportion of the population Hb S disease has been found *e g* in Africa (Foy Kondi and Brass 1951 J and C Lambotte Legrand 1950–51 1952 1955a b Edington 1953 Vandepitte 1954 Trowell Raper and Wellbourn 1957) in Macedonia (Veras Démétriadès and Manios 1953) and in Upper Assam (Dunlop and Mozumder 1952)

The papers of J and C Lambotte Legrand (1950–51 1952 1955b) Vandepitte (1954) and Trowell Raper and Wellbourn (1957) together provide a comprehensive picture of the natural history and high mortality of the disease in Central Africa The Lambotte Legrands found that half of their 300 affected infants died within the first year of life and considered that the fraction reaching adult life was minute while Vandepitte observed only two adults in 244 cases Diagnosis is usually possible by the 4th to 6th month of life Before this time it seems that the presence

The pathological lesions described above presumably follow sickling of the erythrocytes This means that a major degree of oxygen unsaturation must occur somewhere in the renal circulation Whether this can happen in a strictly normal kidney is uncertain possibly ptosis of the kidney or other mechanism leading to partial obstruction to the blood supply are important accessory factors

Hyposthenuria or inability to concentrate urine to the normal degree is another (unexpected) symptomless accompaniment of the presence of Hb S This is referred to later (p 257)

Splenic Infarction While splenic infarction is probably much more easily produced in patients with Hb S/Hb C disease or Hb S/thalassæmia it seems clear that high altitude flying in a non pressurized or an insufficiently pressurized aircraft can give rise to hypoxic sickling of the erythrocytes within the spleen and consequent congestion and infarction in subjects with the simple Hb S trait Severe left sided abdominal pain and nausea are the clinical result

The clinical histories of patients who probably had the Hb S trait only and who developed splenic infarction were described by Cooley Peterson and Jernigan (1954) and by Conn (1954)[1] Smith and Conley (1955) reinvestigated 15 patients who had had splenic infarction during aeroplane flights and found that twelve of them had the Hb S trait Smith and Conley concluded nevertheless that the incidence of infarction was low when the frequency of the trait was taken into account

Further examples of splenic congestion (? infarction) in Hb S trait patients severe enough to produce symptoms have been reported by Rotter and co workers (1956)

Experimentally Levin and his colleagues (1957) obtained support for the idea that moderate degrees of hypoxia could lead to sickling of Hb S trait erythrocytes *in vivo* and their sequestration in the spleen They found by in vivo counting after labelling the erythrocytes of four subjects with ^{51}Cr that there was a significant increase in radioactivity over the spleen following exposure to hypoxia equivalent to a 7 000–8 000 ft altitude

Blood Picture The Hb S trait although leading to a positive sickling test (see p 299) is not associated with anæmia or any definite hæmatological abnormality (Table 12) Blood films appear normal It has been found too that the erythrocytes of healthy carriers of the trait have a normal life span *in vivo* (Singer Robin *et al* 1948 Callender *et al* 1949)

It must of course be realized that carriers of the trait are just as likely to suffer from quite unconnected blood diseases as strictly normal subjects The failure to appreciate this may lead to

[1] The negro referred to in the paper which is usually quoted as being the first in which splenic infarction during an aeroplane flight was reported (Sullivan 1950) probably suffered from Hb S/Hb C disease

Diggs (1956) in a detailed review of the clinical histories of 166 patients with Hb S disease concluded that there was no evidence that the crises were associated with an increase in the severity of the patients anæmia or consistent changes in serum bilirubin urobilinogen excretion or reticulocyte count Increased hæmolysis can thus be ruled out as a common cause of crises (but see above under Hb S disease in infants in Central Africa) An aplastic crisis (see p 263) is clearly also rare this was observed by Diggs only once

Thromboses are also probably responsible for localized foci of aseptic necrosis of bone particularly affecting the head of the femur (Tanaka Clifford and Axelrod 1956 Rowe and Haggard 1957 Carrington Ferguson and Scott 1958) Pain and obvious deformity may develop but some patients have no symptoms although typical sclerosis and bony deformity may be visible on X ray

Osteomyelitis Another interesting and characteristic complication is the increased susceptibility of children with Hb S disease to osteomyelitis Thromboses in the bone marrow leading to infarction probably provide a suitable site for the settling down of organisms absorbed from the gut Various species of *Salmonella* have in fact most commonly been isolated (Diggs Pulliam and King 1937 Vandepitte *et al* 1953 Hughes and Carroll 1957 Hook *et al* 1957 Roberts and Hilburg 1958)

Pulmonary infarcts presumed to be secondary to venous thrombosis or embolism not infrequently occur in the older subjects with Hb S disease they appear to be the pathological basis of cor pulmonale which may sometimes develop (Yater and Hansmann 1936 Klinefelter 1942 Moser and Shea 1957)

A rare complication is bone marrow and fat embolism secondary to bone marrow infarction Shelley and Curtis (1958) described its occurrence in two patients with Hb S disease [1] and in a further patient with Hb S/Hb C disease The initial complaint of bone pain was followed by mental depression pyrexia and the appearence of skin petechiæ Two of the patients were pregnant Shelley and Curtis suggested that bone marrow embolism may be the pathological basis of some of the instances of sudden death in Hb S disease Five other similar cases were found in the literature

Surgical operations in patients with sickle cell disease present a special hazard Leaving aside diagnostic difficulties such as the distinction between intra abdominal inflammation and abdominal

[1] The patient described by Wade and Stevenson (1941) was probably suffering from Hb-S/thalassæmia

of a high concentration of fœtal hæmoglobin (and a consequent low concentration of Hb S) protects (see p 299)

According to Trowell Raper and Wellbourn (1957) although Hb S disease may be suspected at the 3rd month anæmia is not usually marked until the 5th to 6th month By the 7th to 15th month severe anæmia is almost invariably present Subsequently there is a tendency for the anæmia to be less severe A characteristic clinical sign in infancy is recurrent swelling of the hands and feet apparently due to periosteal thickening—this had also been noted by Watson (1956) in negro infants in America The spleen is moderately enlarged and usually palpable but evidence of thrombosis and ulceration of the leg are not commonly seen Episodes of increased hæmolysis occur quite frequently in the second 6 months of life and are accompanied by jaundice which otherwise is inconspicuous these episodes may lead to the death of the patient Unexplained pyrexia occurs quite frequently

In America most of the studies have been carried out on older patients According to Wintrobe (1956) only twelve cases in infants up to the age of 1 year had been recorded up to the time of writing and Scott and co workers (1955) reported that the mean age at first admission into hospital of 63 negro children with Hb S disease was 4 7 years—their ages ranged from 4 months to 15 years Leikin and McCoo (1958) have however recently described Hb S disease in two infants aged 24 days and 6 weeks respectively

The mortality in America is clearly far less than it has been in Africa and the same is true of the negro population of the West Indies Went and MacIver (1958b) for instance found that 19 patients out of a total of 114 were 26 years of age or older

In older children and in adolescents and adults the disease usually runs a fairly stable course the main symptoms are those attributable to chronic anæmia *i e* weakness and dyspnœa From time to time however exacerbations take place and at these times it is usual for the patients to complain of aching pains in the joints or elsewhere in the limbs and sometimes of abdominal pain and nausea These crises are usually associated with pyrexia Comprehensive accounts of the symptomatology of Hb S disease are given in many publications *e g* those of Sydenstricker (1924) Grover (1947) Smith and Conley (1954) and Wintrobe (1956) Margolies's (1951) review is a most valuable source of information on the earlier literature

Thrombotic Crises The crises of Hb S disease are of great clinical importance for they may closely mimic acute rheumatism or an acute abdominal catastrophe They probably arise as a consequence of intravascular agglutination of sickled cells leading perhaps to local thrombosis and tissue ischæmia

coma or hemiplegia (the most frequent symptoms) to marked salivation or delirium (the least frequent)

The *radiographic findings* in Hb S disease have been extensively studied Excluding evidence of cardiac enlargement the most important changes are found in the bones (Caffey 1937 Diggs Pulliam and King 1937 Golding 1956 Middlemiss 1958) The main abnormalities are irregularity of the bony trabeculæ with widening of the medullary cavities thickening of the diploe of the skull and areas of sclerosis On the other hand osteoporosis may develop and rarely spontaneous fractures The occurrence of aseptic infarcts leading to pain and deformity has already been referred to

Diggs Pulliam and King (1937) pointed out that two opposing processes take place within the bones and are responsible for the X ray changes widening of the medullary spaces due to hyperplasia of the marrow and narrowing of the spaces due to sclerosis Occasionally areas of calcification in the spleen can be seen (Caffey 1937 Ehrenpreis and Schwinger 1952)

Urine An unexpected finding in Hb-S disease and in the trait is impairment of the power to concentrate urine (hyposthenuria) This abnormality has been extensively studied recently (Kunz *et al* 1953 McCrory Goven and Gornfeld 1953 Kunz *et al* 1954) It is however no new observation for Sydenstricker (1924) remarked The urine of both types of case [latent and active cases of sickle-cell anæmia] has a low specific gravity Keitel Thompson and Itano (1956) in an extensive study found that in young patients with Hb-S disease the defect in concentration could be reversed after multiple transfusions They concluded that the abnormality was in some as yet unknown way associated with the presence of Hb S (the formation of which would be depressed by transfusion) it was not due to anæmia itself as 69 % of the patients with Hb-S trait studied also had the defect although it was less marked Etteldorf Smith Tuttle and Diggs (1955) carried out renal hæmodynamic studies on patients with Hb-S disease they found that renal function became progressively impaired as the patients became older

The *eyes* have been reported as being affected with a variety of pathological changes in patients with Hb S disease in particular tortuosity and dilatation of the retinal veins (see Margolies 1951 Hannon 1956) Henry and Chapman (1954) and Hannon (1956) discussed the frequency of retinal complications Vitreous hæmorrhages are rarely met with in Hb S disease and they are also rare in Hb-S trait although they have been observed (Isbey and Clifford 1958) They seem, however to be relatively much more frequent in Hb-S/Hb-C disease (see p 269)

Pregnancy in Sickle Cell Disease and Sickle Cell Trait

As already mentioned it is unusual for a subject with Hb S disease to survive until adult life Nevertheless this does happen

(thrombotic) crises anæsthesia itself may be harmful Particular care must be taken to avoid hypoxia and hypotension (Shapiro and Poe 1955)

Clinical Signs Physical examination reveals a pallor of the mucous membranes and typically a greenish yellow colour of the conjunctivæ (Margolies 1951) Smith and Conley (1954) remarked on the asthenic build of their patients and the disproportionate length of their legs

Chronic ulcers of the leg are commonly found, according to Chernoff Shapleigh and Moore (1954) 30–50% of patients are affected The ulcers are usually bilateral and lie superficial to or just above the internal or external malleoli They are more frequently found in adolescents and adults than in children

The *spleen* is usually palpable in children but this is by no means invariable it is not usually palpable in adults (see Pathology p 260) Watson Lichtman and Shapiro (1956) reported that the spleen was palpable in 18% of 115 patients 33% of those in the first decade had palpable spleens and 10% of those who were older As will be referred to later splenomegaly is more frequent and persists into adult life in Hb S/Hb C disease and Hb S/thalassæmia

The *liver* is frequently palpable particularly in children (Margolies 1951 Green Conley and Berthrong 1953) According to Green Conley and Berthrong there is often clinical evidence of *hepatic dysfunction Gallstones are found in about one* third of the patients (Weens 1945 Green *et al* 1953 Jordan 1957)

Enlargement of the *heart* chiefly affecting the right side is common and according to Klinefelter (1942) this is often more severe than is usually found in other chronic anæmias of comparable severity As already mentioned cor pulmonale sometimes develops

Cardiac output was studied by Leight and co workers (1954) and was found to be generally elevated although the increase did not seem to be proportional to the degree of anæmia present Electrocardiographic findings were described by Lindo and Doctor (1955) Blood gas determinations have been carried out by Becklake and co workers (1955) Fowler Smith and Greenfield (1957) and Sproule Halden and Miller (1958) As a group patients with Hb S disease were found to have low arterial oxygen tensions and increased alveolar-arterial oxygen gradients The dissociation curve of oxyhæmoglobin was displaced to the right of the normal

The *central nervous system* may be involved in Hb S disease as a result of thromboses occurring in the cerebral vessels The symptoms and signs are extremely variable Margolies (1951) listed 28 different symptoms ranging from drowsiness stupor

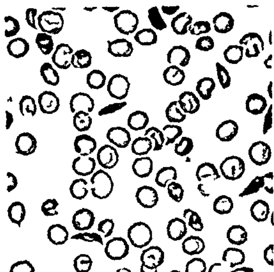


Fig. 100 Photomicrograph of a blood film of a child with Hb S disease. A small number of sickled cells are present. ×700



Fig. 101 Photomicrograph of a blood film of a child with Hb S disease. Many sickled cells are present, an unusual finding. × 700

To face p. 3

sometimes and if pregnancy ensues this is a serious event The patient s anæmia becomes aggravated and there is an increased risk of toxæmia and infection Obstetric complications appear to be frequent and the mortality risk to the mother and child is high The earlier literature was reviewed by Margolies (1951) who stated that only 50 instances of pregnancy had been reported up to the time of his review The first comprehensive reports were those of Kobak Stein and Daro (1941) and Beacham and Beacham (1950) Later papers include those of Adams Whitacre and Diggs (1953) and Eisenstein Posner and Friedmann (1956)

According to Margolies (1951) the presence of the Hb S trait does not affect conception or increase the likelihood of complications in pregnancy Adams Whitacre and Diggs (1953) however considered that pregnancy was in fact a hazard and that both the incidence of toxæmia and the fœtal mortality were increased Any such hazard is clearly far less than that attending pregnancy in Hb S disease or Hb S/Hb C disease (see p 268)

The Blood Picture in Sickle Cell Disease (Sickle Cell Anæmia)

Erythrocytes Anæmia is moderate or severe the erythrocyte count is usually between 2 000 000 and 3 500 000 cells per cu mm but it may fall as low as 1 500 000 per cu mm The MCV is generally normal occasionally in the most anæmic cases it is above normal The MCHC is also usually normal (Diggs and Bibb 1939 Itano Bergren and Sturgeon 1956) (Table 12 p 266)

In stained films anisocytosis is moderate in extent and many of the cells appear hypochromic As a rule a few conspicuously *elongated cells with sharp or rounded ends are present* some may be oat shaped or sickle shaped (Fig 100) Sometimes target cells are present Occasionally numerous sickled cells can be seen (Fig 101) Polychromasia is often marked the cells staining diffusely basophilic being usually round in contour The reticulocyte count is typically raised and usually ranges between 5% and 20% Normoblasts are often present in small numbers Moderate numbers of siderocytes may be found in peripheral blood films even before splenectomy (Kaplan Zuelzer and Neel 1953)

Erythrocyte osmotic fragility is usually moderately diminished but there may be a small tail of unusually fragile cells (Fig 20 p 38) On incubation at 37 C for 24 hours the majority of the erythrocytes become still more resistant but at the same time the proportion of unusually fragile cells increases The results are

generally the same whether or not the actual tests are carried out on oxygenated or deoxygenated blood (Harris *et al* 1956)

Erythrocyte mechanical fragility is significantly raised above the normal when Hb S disease blood equilibrated with room air is tested When deoxygenated the increase in fragility is most marked (Harris *et al* 1956)

Leucocytes The leucocyte count may be raised to 20 000 cells per cu mm or more when hæmolysis is active The leucocytosis is chiefly due to an increase in the polymorphonuclear neutrophils a few myelocytes may also be present A neutrophil leucocytosis usually also occurs in 'thrombotic crises and when these are characterized by abdominal pain the raised leucocyte count may lead to the misdiagnosis of intra abdominal inflammation

Platelets The platelet count is usually normal

Plasma Bilirubin The plasma bilirubin level is usually moderately increased as a rule to 1–2 mg per 100 ml

Plasma Proteins According to Fenichel Watson and Eirich (1950) abnormalities in the plasma protein pattern are frequently found Thirteen out of 15 patients with Hb S disease had decreased concentrations of albumin twelve had elevated γ globulin concentrations and three raised concentrations of β globulin The plasma fibrinogen level was high in eight out of ten of these patients Fenichel and co-workers suggested that these changes might be non specific reactions secondary to tissue breakdown and that this occurred particularly in the liver as the result of vascular obstruction due to sickling

Similar studies have been carried out by Allamanis (1955) In infants less than 2 years of age the plasma protein patterns were usually normal In older children the concentrations of albumin and α_1 α_2 and β globulins were found to be decreased and that of γ globulin to be increased

Choremis Zannos and Basti (1957) studied the serum and urinary amino-acids in Hb S disease As in Cooley s anæmia the serum amino-acid concentrations appeared to be diminished in the urine however the number and concentrations of demonstrable amino acids were markedly increased

Electrophoresis of Hæmoglobin The majority (80–100%) of the hæmoglobin in Hb S disease is Hb S with a varying but usually small proportion of Hb F Hb A is absent (Pauling *et al* 1949 Wells and Itano 1951) This Hb-S plus Hb-F pattern although very suggestive of (homozygous) Hb S disease is not absolutely diagnostic of it as Hb A may be absent sometimes in apparently true cases of Hb S/thalassæmia (see p 267)

Bianco (1948) seems to have been the first to have demonstrated an increased resistance to denaturation by alkali of the hæmoglobin of carriers of the sickling trait (falcemia) and of one patient with microdrepanocytic disease Subsequently in America following the

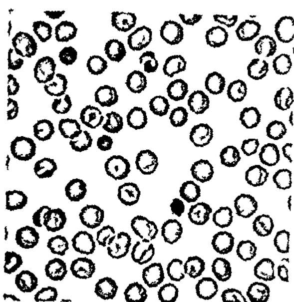


Fig. 102. Photomicrograph of a blood film of a woman with Hb S/Hb C disease. Numerous target cells and occasional spherocytes are present. No sickled cells are to be seen. × 700

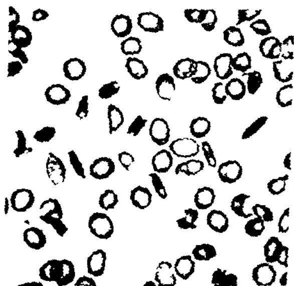


Fig. 103. Photomicrograph of a blood film of a child with Hb S/Hb D disease. Moderate numbers of elongated oat-shaped cells are present (Case 7 of Table 19.4). × 700

for the patient's anæmia. In films of bone marrow made *post mortem* filiform erythrocytes of up to 40 μ in length were noted.

Another relatively early and detailed account is that of Diggs and Ching (1934). This was based on necropsies carried out on four patients with severe Hb S disease and 30 patients with Hb S trait.

A recent account is that of Edington (1955), who reviewed the findings in 40 necropsies carried out at Accra. Thirteen of the necropsies were on pregnant patients who had died suddenly. Not all Edington's patients were homozygous for Hb S; some certainly had Hb S/Hb C disease. Nevertheless his report contains much of interest. The pathological findings could be grouped into two categories: those associated with hæmolysis, *e.g.* jaundice and siderosis, and those due to thromboses leading to infarction of the lungs, spleen and elsewhere. In five of Edington's patients the spleen was reduced to a small fibrous mass (siderofibrotic spleen)—the classical finding in Hb S disease; in others it was congested. In pregnant patients who died severely anæmic the pallor of their organs contrasted sharply with the dark red blood of the fœtus.

Microscopic Findings

Bone Marrow. Erythropoietic cells predominate; fat cells tend to disappear. Erythropoiesis is typically normoblastic. However, as in other hæmolytic anæmias and in Mediterranean anæmia, megaloblastic transformation of the bone marrow might be expected to occur from time to time. Walters and Young (1954) have in fact reported an example of this in a young Arab woman (with Hb S/thalassæmia) during pregnancy.

Sickled cells are usually more conspicuous in marrow than in the peripheral blood. As mentioned previously, Sydenstricker, Mulherin and Houseal (1923) mentioned the presence of long filiform erythrocytes in post mortem films. Recently, Vandepitte and Louis (1953) have called attention to the presence of similar structures in wet preparations of biopsied bone marrow in Hb S disease, but not in the trait.

Spleen. The contracted spleen of Hb S disease results from a more or less complete replacement of its normal structure by iron-impregnated fibrous tissue. Crystals of calcium salts may be present in addition. In enlarged congested spleens, as in young subjects with Hb-S disease and in Hb S/Hb C disease, the spleen pulp is stuffed with enormous numbers of very tightly packed sickled cells and the sinusoids are compressed to tiny chinks in a sea of blood (Edington, 1955). Perifollicular hæmorrhages are sometimes a conspicuous feature. Erythrophagocytosis may be discernible.

Rich (1928) pointed out that "pools of blood partly or completely surrounding the Malpighian bodies" were a characteristic finding in the spleen of patients with Hb S disease or trait, and he interpreted this as being due to a congenital malformation of the splenic sinuses allowing a free escape of blood into the pulp. This concept has not been accepted and it seems now to be generally agreed that the pooling of blood in the spleen pulp, particularly around the periphery of the Malpighian bodies, is due to sickling of the erythrocytes and their consequent inability to escape through the stomata into the venous sinuses of the spleen (Tomlinson, 1945).

discovery of Hb S detailed measurements of fœtal type hæmoglobin were carried out by Singer and his collaborators (Singer Chernoff and Singer 1951 Singer and Chernoff 1952 Singer and Fisher 1952 1953) Itano (1952) and Chernoff (1953)

As in Mediterranean anæmia the conclusion was reached that the fœtal type hæmoglobin present was in fact identical with normal Hb F (Itano 1953b Chernoff 1953)

Singer and Fisher (1952) reported the results of an analysis of 87 patients who gave unquestionable clinical and hæmatological evidence of sickle cell disease the erythrocytes of three patients contained no Hb F by the method used in the others the proportion ranged from 2 to 24% They concluded as the result of transfusion experiments that *the erythrocyte population of patients with sickle cell disease was* probably composed of three fractions (1) cells containing Hb S but little or no Hb F (2) cells containing both Hb S and Hb F and (3) cells containing Hb F with little or no Hb S The corpuscles containing Hb S had the shortest survival when transfused to normal recipients and the greatest sensitivity to mechanical trauma *in vitro* and the cells containing the most Hb F the longest survival *in vivo* and the greatest resistance to trauma *in vitro*

Attempts have been made to differentiate between Hb S and Hb A by immunological means One of the first attempts was carried out by Cardozo (1937) rabbits were immunized with blood containing sickle cells and the rabbit sera subsequently absorbed with normal erythrocytes No specific agglutinins for sickle cells however could be demonstrated Vecchio and Barbagallo (1950) immunized rabbits with different types of hæmoglobin Using the precipitin reaction they failed to demonstrate any antigenic difference between Hb A and Hb S however they did find a difference between Hb A and Hb F as had been previously demonstrated by Darrow Nowakovsky and Austin (1940) Chernoff's (1953) results were essentially the same as those of Vecchio and Barbagallo Goodman and Campbell (1953) on the other hand using anti sera prepared in chickens reported that clear differences in the antigenicity of Hb A and Hb S could be demonstrated if cross reaction tests were carried out quantitatively under optimum conditions They suggested that the two different hæmoglobins share many common antigenic determinants but that a small number are unique for each type

Pathology of Sickle Cell Disease

The usual findings are signs of anæmia and of cardiac failure hepatomegaly and hæmosiderosis The spleen is characteristically small and fibrotic but may be enlarged in young children and *there is often evidence of infarction of other organs* The bone marrow is hyperplastic and increased in extent

The first published record of the findings *post mortem* in a case of Hb S disease appears to be that of Sydenstricker Mulherin and Houseal (1923) This is a remarkably good account the small size of the spleen was mentioned and was regarded as the consequence of repeated hæmorrhages The distribution of many brown granules in the liver and kidney was thought to be strongly in favour of a hæmolytic origin

secondary to impaction of sickled erythrocytes Kimmelstiel (1948) made the point that widespread infarction may occur—in his case of the brain liver and kidneys—without there being histological evidence of thrombus formation

Aplastic Crises in Sickle Cell Disease

In 1950 Singer Motulsky and Wile described two children suffering from Hb S disease in whom there was an abrupt increase in the severity of their anæmia This was found to be associated with reticulocytopenia and temporary cessation of erythropoiesis in the bone marrow In both instances the crisis seems to have been precipitated by infections The course of events appears to have been the same as in aplastic crises in hereditary spherocytosis (see p 110) In 1951 Chernoff and Josephson reported four more instances of aplastic crisis in Hb S disease in three patients the initiating cause seemed to be an upper respiratory tract infection and in one patient infection with *Salm choleræ suis*

Further crises have been reported by Leikin (1957) and Miesch Baxter and Levin (1957) Leikin s cases occurred in two families No exciting cause was discernible but in both families two children were affected successively within a 2 week period One further case was referred to In each patient there was marked marrow erythroblastopenia at the height of the crises but not leucopenia or thrombopenia

Aplastic crises are clearly unusual events in Hb S disease This is underlined by the extensive data of Diggs (1956) who reviewed the findings in 166 patients admitted to hospital 747 times with clinical crises Only one of these admissions was for a true aplastic crisis

COMBINATIONS OF HÆMOGLOBIN S WITH THALASSÆMIA OR OTHER ABNORMAL HÆMOGLOBINS

At a time when Hb S was the only abnormal hæmoglobin that had been discovered cases of sickle cell anæmia were known in which only one of the parents of the propositus could be shown to be a carrier of the sickle cell trait Neel (1952) suggested that the most likely explanation was that the normal parents had contributed another gene (or genes) which in combination with a single sickle cell gene produced overt sickle cell anæmia In most instances at least this has been shown to be the correct explanation and many patients in whom the gene for Hb S has been combined with that for thalassæmia or Hb C have been studied as well as a

Liver Major pathological changes are not uncommon Green Conley and Berthrong (1953) reported on 21 necropsies unequivocal cirrhosis was found in four patients and in many of the others there were active or healed areas of necrosis The necrosis was thought to be due to vascular obstruction brought about by impacted masses of sickled cells or by Kupffer cells swollen with phagocytosed erythrocytes

Further details were given by Song (1955 1957) Song (1957) reviewed 31 necropsies Some evidence of liver damage was present in all The lesions were interpreted as being due to impairment of the circulation leading to stagnation of sickled cells to the formation of thrombi composed of agglutinated and hyalinized sickled cells and ultimately to anoxic necrosis of liver cells The liver sinuses were generally dilated and the parenchyma showed various degrees of necrosis atrophy siderosis and fibrosis The degree of fibrous tissue proliferation justified the diagnosis of cirrhosis in nine instances

Rogoch and co workers (1955) reported on the results of needle biopsy in four patients evidence of hepatitis hæmochromatosis or portal cirrhosis was observed

Edington (1955) in his study of 40 cases in West Africa reported only one instance of cirrhosis However increased fibrosis was noted in two other patients central atrophy in two patients and focal necrosis in one The parenchymal cells were generally relatively unaffected The most striking feature was erythrophagocytosis by Kupffer cells some of which were distended with as many as 20–30 sickled erythrocytes The sinusoids were generally dilated and congested and in four instances a fibrin network was present in the sinusoids in which sickled erythrocytes were enmeshed Areas of extramedullary erythropoiesis were noted in eight instances It is interesting to note that Edington observed in three necropsies in which the spleen was enlarged and engorged with blood that there was no evidence of erythrophagocytosis in the liver

Walters (1958) has recently reported on the liver biopsy studies which he carried out in Nigeria The most striking finding present in every specimen was wide dilatation of the sinusoids with the presence within their lumen of a delicate foam like reticulum Walters considered that the reticulum was composed of fibrin it appeared to be in continuity with the longitudinal fibres of the sinusoidal walls and in time to be incorporated into them Walters suggested that this process of *intrasinusoidal deposition of fibrin could explain the appearances seen* in advanced cases the liver cells being separated by a wide zone of coarse fibrous tissue from the endothelial wall of patent though narrowed sinuses

These observations are of great interest It is certainly possible that sickled erythrocytes when impacted liberate thromboplastic material locally Indeed McKellar and Dacie (1958) obtained evidence in three cases of sickle cell disease that incubation *in vitro* of their whole blood at 37 C led to a considerable rise in the thromboplastic activity in their plasma This increased activity was independent of the presence of platelets or the onset of hæmolysis

Kidneys Edington (1955) described congestion of the glomerular tufts This appears to lead to thrombosis for partially or completely fibrosed glomeruli were not infrequent

Other Organs The basic changes leading to infarcts in the lungs or cerebral softening appear to be occlusion of small blood vessels

concluded that the disease might occur in quite mild forms for one of their patients was not anæmic although his blood film was characteristic Silvestroni and Bianco (1955) gave further details of the disease as it occurs in Sicily They too stressed that it may be quite a mild disorder and may not be clinically manifest until late childhood Osteo-articular crises and leg ulcers were infrequent in their patients and anæmia was seldom severe the hæmoglobin range recorded being 50–72% A leucocytosis was frequently present The mortality however appeared to be high and only two of the patients were adults Some of the children showed signs of physical retardation and Silvestroni and Bianco made the point that successful pregnancies seemed to be very rare amongst adult women with the syndrome

In South Turkey Hb S/thalassæmia is not infrequent among the Eti Turks (Aksoy and Lehmann 1957a) Amongst this population too the severity of the disease seems to vary greatly for in addition to five anæmic patients two individuals were discovered who were heterozygous for the Hb S and thalassæmia genes but who were not anæmic and were symptom free Aksoy and Lehmann pointed out that as is shown by the variable expression of the gene for thalassæmia in thalassæmia minor the clinical effect of being heterozygous for Hb S and thalassæmia probably depends upon the degree to which the thalassæmia gene is able to suppress Hb A formation

As already indicated patients with Hb S/thalassæmia may suffer from the consequences of intravascular sickling and their symptoms are thus similar to those of patients with (homozygous) Hb S disease although generally more variable in intensity and usually less severe This applies also to the less common symptoms thus the subject of the first well authenticated report of fat embolism derived from necrotic bone marrow was a Greek woman aged 49 years who probably had Hb S/thalassæmia (Wade and Stevenson 1941) Splenic infarction as a result of an aeroplane flight (Rotter *et al* 1956) and *Salmonella* osteomyelitis (Silver Simon and Clement 1957) have also been reported

The findings at necropsy in a case of microdrepanocytic disease were reported in detail by Ascenzi and Silvestroni (1957)

Blood Picture Hb S/thalassæmia is characterized by a hypochromic anæmia of variable severity markedly decreased erythrocyte fragility microcytosis and striking anisopoikilocytosis with many oval cells and target cells (Table 12) Sickle cells are not as a rule visible in freshly made blood films but sickling can be induced *in vitro* The optical properties of the sickled corpuscles are the same as those in the Hb S trait (Ascenzi and Silvestroni 1953) Went and MacIver (1958b) have pointed out that the presence of marked hypochromia numerous target cells and but few sickle cells is suggestive of Hb S/thalassæmia rather than of Hb S disease

small number in whom the gene for Hb S has been combined with that for Hb D or Hb E Combinations of Hb S with other abnormal hæmoglobins no doubt exist but they seem likely to be rare and may not produce characteristic clinical or hæmatological effects

The combinations of Hb S with thalassæmia Hb C or Hb D are important for they lead to clinically significant hæmolytic syndromes

Sickle Cell Hæmoglobin/Thalassæmia (Hb S/Thalassæmia) or Microdrepanocytic Disease One Gene for Hb S and One Gene for Thalassæmia

This form of sickle cell disease was described by Silvestroni and Bianco (1944–45 1946 1952) as la malattia micro drepanocitica Subsequently several American families of Italian Sicilian or Greek origin were found to be suffering from the same syndrome (Powell Rodarte and Neel 1950 Banks Scott and Simmons 1952 Wasserman Phelps and Hertzog 1952 Sturgeon Itano and Valentine 1953 Neel Itano and Lawrence 1953) Silvestroni and Bianco (1952) stated that microdrepanocytic disease could be distinguished from true Hb S disease by hæmatological characteristics as well as by genetical studies

Later reports have shown that the syndrome has a wide *distribution outside Italy and the United States it has been* observed for instance in Kuwait (Walters and Young 1954) in Tunisia (Roche *et al* 1956) in South Turkey (Aksoy and Lehmann 1957a Aksoy 1959) in Greece where it is widely distributed (Choremis and Zannos 1957) in Jamaica (MacIver Went and Cruickshank 1958 Went and MacIver 1958a) and in India (Chatterjea *et al* 1958 Chatterjea 1959)

Clinically the disease is usually a serious one although many patients reach adult life Typically as in true Hb S disease the patient presents with chronic anæmia slight to moderate jaundice hepatosplenomegaly chronic ulcerations of the leg recurrent bouts of fever osteo articular pains and sometimes with crises of severe abdominal pain

Humble and co workers (1954) reported some interesting consequences of the marriage of a negro with Hb S trait and a Caucasian from Naples with thalassæmia minor One of the children thought to be a double heterozygote *i e* to have Hb S/thalassæmia was anæmic with signs of hæmolysis however two other brothers also apparently double heterozygotes were not anæmic and showed no signs of hæmolysis

Singer Singer and Goldberg (1955) reported the occurrence of Hb S/thalassæmia in *four female negroes in America They also*

Electrophoresis of Hæmoglobin Hb S Hb F and Hb A are typically present together The exact proportions differ from case to case and occasionally Hb A may be absent In such cases the differentiation from Hb S disease may be difficult and perhaps only satisfactorily settled by family studies (see Singer *et al* 1957) According to Zuelzer Neel and Robinson (1956) the usual finding is for 60–80% of the hæmoglobin to be Hb S Hb F is not always present the maximum recorded amount being 17% Later observations have however indicated that the proportion of Hb S may be much less than was at first regarded as typical and Zuelzer Neel and Robinson mention three patients having from 22–36% of Hb-S who were essentially symptom free

Singer and his co-workers (1957) have argued that the wide variation in the amount of Hb S that may be present supports the contention that there is more than one form of thalassæmia gene which differ in their capacity to suppress Hb-A formation or alternatively that modifying genes are responsible Gene interaction similarly appears to take place in Hb-C/thalassæmia where the proportion of Hb C is usually in the range 60–80 % instead of the theoretical 50% (see p 277)

A similar argument has been put forward by Went and MacIver (1958b) who have related the different hæmoglobin patterns which may be found to the racial origin of the patients They reported that in three Afro Chinese subjects Hb-A was absent and there was 10 % of Hb-F while in eight Afro-Caucasian or African subjects there was 15–27 % of Hb A and only small amounts of Hb F

Syndromes Apparently Allied to Hb S/Thalassæmia

As mentioned previously (p 215) there are several reports which suggest that a gene exists whose effect is to permit the persistence into adult life of large amounts of Hb-F unassociated with anæmia or any hæmatological abnormality In combination with Hb S the double heterozygotes resemble Hb-S disease electrophoretically but again there is no anæmia and the bearers of the combination do not complain of any symptoms

Edington and Lehmann (1955) reported two normal adults whose hæmoglobin on electrophoresis gave patterns resembling homozygous Hb-S disease in that the only hæmoglobins present were Hb S and Hb-F They concluded that they were dealing with the combination of Hb S and a thalassæmia like gene Neel and co-workers (1956) also came across in West Africa three apparently healthy men whose hæmoglobin also appeared to consist only of Hb S and Hb-F Further studies were reported by Jacob and Raper (1958) from Uganda Four Africans without anæmia were found to have a hæmoglobin pattern similar to that of homozygous Hb-S disease Examination of two of the families appeared to exclude thalassæmia but it revealed the presence of Hb-F in relatively high proportion (25 % and 12 % respectively) in the blood of one of the parents of each of the two propositi Jacob and Raper concluded that a gene exists which is only just beginning to be

Table 12

Hæmatological findings in the Abnormal Hæmoglobinopathies

Disorder	Erythrocytes (mill / cu mm)	Hæmoglobin (g /100 ml)	MCV (cu μ)	MCHC (%)	Reticulocytes (%)	Target cells (%)	Abnormal hæmoglobins (%)	Foetal hæmoglobin (%)	Sickling (+ or –)
Normal (adult men and women)	3 9–6 5	11 5–18 0	76–96	32–36	0 2–2 0	0	0	0	–
Hb A/Hb S	N	N	N	N	N	0	2[illegible]–48 (Hb S)	0–trace	+
Hb-S/Hb S	1 5–4 0	2–11	72–100	30–36	5–30	Some	80–100 (Hb S)	0–20	+
Hb S/thalassæmia	2 0–5 0	6–14	65–90	25–35	4–20	Many	22–80 (Hb S)	0–17	+
Hb S/Hb C	2 5–5 5	8 1–15 1	65–90	28–34	0 2–10	5–85	37–67 (Hb C) 30–60 (Hb S)	0–8	+
Hb S/Hb-D	2 5–4 0	7–1[illegible]	100–118	30–32	7–13	2–Some	? 50 (Hb S) ? 50 (Hb D)	Trace	+
Hb S/Hb E	4 8–5 6	11 4–13 2	93–95	25		Some	40 (Hb E) 60 (Hb S)	?	+
Hb A/Hb C	N	N	N	N	N	0–40	25–39 (Hb C)	0	–
Hb C/Hb C	3 1– 0	7–14 5	55–93	23–38	1–12	20–100	97–100 (Hb C)	0–4	–
Hb C/thalassæmia	4 6–5 4	4 1–12 7	50–67	22–35	2–7	[illegible]0–60	[illegible]9–93 (Hb C)	Trace–3	–
Hb A/Hb D	N	N	N	N	1–2 8	0	<50 (Hb D)	0	–
Hb D/Hb D	5 5–7 1	12–13	63–67	28–35	1–1 5	50–80	100 (Hb D)	0	–
Hb A/Hb E	N	N	N	N	N	Few	20–50 (Hb E)	0	–
Hb E/Hb E	4 0–8 4	8 8–16 3	51–76	27–35	0 4–2	9–70	94–100 (Hb F)	Trace–6	–
Hb E/thalassæmia	1 3–4 2	[illegible] 3–8 5	61–83	24–32	1–9 5	4–44	15–45 (Hb E)	55–85	–
Hb H/thalassæmia	1 0–6 4	2 7–11 3	49–113	17–30	2–22	1–30	35–40 (Hb H)	Trace–37	–

The above data are taken from the various papers quoted in the text They illustrate the wide range of variability in the blood pictures of patients apparently suffering from the same disorder
N = within normal range

also been recorded (Vandepitte and Colaert 1955 Lohmuller and Marshall 1958 Smith and Krevans 1959) Smith and Krevans (1959) mention three instances of unexplained hæmaturia

Eye complications appear to be relatively frequent in particular vitreous hæmorrhages (Hannon 1956 Goodman von Sallmann and Holland 1957 Isbey and Clifford 1958) Goodman von Sallmann and Holland pointed out how relatively frequently the eyes and kidneys are both involved in systemic diseases and instanced hypertension diabetes and the sickle cell syndromes

X ray Findings Cockshott (1958) has recently described the radiological appearances in 20 patients Slight changes in the bony texture of the skull and long bones were noted in about one third of the patients but these did not seem to be related to age or to the degree of anæmia Evidence of bone infection was frequently found Avascular changes were noted also in about one third of the cases the hips and shoulders being most affected The lesions were multifocal discrete and subchondral

Hæmatological Findings The blood picture differs both qualitatively and quantitatively from that of Hb S disease The blood sickles *in vitro* in much the same way as does blood from Hb S disease but sickled cells are rare in films of fresh peripheral blood Anisocytosis and poikilocytosis are not conspicuous but target cells are usually present in large numbers up to 85% according to Zuelzer Neel and Robinson (1956) (Fig 102)

Anæmia is slight to moderate in severity as a rule and Smith and Conley (1954) found that the PCV exceeded 30% in all their cases Went and MacIver (1958b) reported the mean hæmoglobin level of 45 patients to be 11 1 g per 100 ml (range 8 1–15 1 g per 100 ml) According to Itano Bergren and Sturgeon (1956) the MCV ranges from 66 to 90 cu μ and the MCHC from 28 to 34% (Table 12 p 266)

The reticulocyte count is only slightly or moderately raised and normoblasts are rare in films of peripheral blood The erythrocyte osmotic fragility is diminished to about the same extent as in true Hb S disease Small numbers of siderocytes may be present The plasma bilirubin level is normal or slightly increased and the fæcal urobilinogen excretion is moderately increased (Kaplan Zuelzer and Neel 1953)

Cook and Cooper (1958) have published some interesting figures in which are compared the hæmatological findings during asymptomatic periods of 16 patients with Hb S/Hb C disease with those of 36 patients with (homozygous) Hb S disease Although the Hb S/Hb C disease patients were generally less anæmic there was some overlap between

recognized which permits the persistence into adult life of Hb F unassociated with any obvious additional hæmatological abnormality

More recently Went and MacIver (1958a) have reported the existence of what is probably the same condition in a Jamaican family of African origin The propositus was a 26 year old female who was pregnant for the fifth time She was not anæmic gave no history of anæmia and had already had four children successfully Her hæmoglobin nevertheless consisted solely of Hb F (27%) and Hb S High values for Hb F were found in three generations of the family unassociated with any of the usual signs of thalassæmia [1]

Sickle Cell Hæmoglobin/Hæmoglobin C Disease (*Hb-S/Hb C Disease*) *One Gene for Hb S and* One Gene for Hb C

The clinical syndrome associated with the combined presence of Hb C and Hb S was first described by Kaplan Zuelzer and Neel (1951) Later reports from the United States include those of Smith and Conley (1953) Neel Kaplan and Zuelzer (1953) *Kaplan Zuelzer and Neel (1953) Smith and Conley (1954)* and Hook and Cooper (1958) Edington and Lehmann (1954a) described the first case to be observed outside America

Hb S/Hb C disease resembles Hb S disease in many respects but it is milder and follows a more benign course Smith and Conley (1954) studied sixteen cases and made a detailed comparison between the clinical features of the two syndromes Although in their series the disorder was often diagnosed in childhood this was not always so and in several patients symptoms did not arise until late adult life Far fewer crises developed than in true Hb S disease Only four patients for instance had recurrent abdominal pains and only seven episodic joint pains Unexplained hæmaturia occurred but rather rarely The liver was enlarged in only three patients but the spleen was palpable in most of them and showed no signs of regression in size as the patients became older

Pregnancy appeared to be a definite hazard and all the five patients studied became severely anæmic during the last trimester or *post partum* Two died of post partum hæmorrhage and one other died for no obvious cause (see also Smith and Krevans 1959)

As already mentioned *infarction of the spleen* during aeroplane flights seems to be a not uncommon event in patients with Hb S/Hb C disease (Motulsky 1954 Doenges Smith and Wise 1954 Colman and Furth 1956 Smith and Conley, 1955) Aseptic necrosis of the head of the femur and bacterial osteomyelitis have

[1] Lehmann (1959b) refers to the result of the presence of the gene as non microcythæmic thalassæmia.

was born in Jamaica as he believed of Irish French English and Scottish ancestors he had nevertheless the Hb S trait The mother was English but she had remote ancestors who were Austrian and Spanish she had the Hb-D trait

Stewart and MacIver s (1956) patient was a woman aged 32 who attended hospital complaining of ulceration of the ankles Her mother was English but she had the Hb-D trait and her father was a negro from West Africa he was dead but presumably had contributed the gene for Hb-S The propositus was anæmic there were 8 7 g of hæmoglobin per 100 ml and the erythrocytes underwent filamentous sickling *in vitro* The patient died of pyelonephritis at necropsy it was noticed that the spleen was extremely small in size

A further case has more recently been described by Smith and Conley (1959) Their patient was a young man of Caucasian appearance who was aged 20 when investigated His ancestors had originally come from England His main complaint was of recurrent episodes of pain in the back and limbs which he had had since childhood The pain was worse at night and exposure to cold in the afternoon tended to bring it on He also had had attacks of pneumonia and transient ulcers of the legs He had a hæmolytic anæmia but this had not been serious enough to stop him being an athlete

Smith and Conley point out that patients with Hb S/Hb-D disease may have long clinical remissions and may even not be anæmic They attributed the bone pains and pulmonary manifestations to thrombosis secondary to occlusion of vessels by sickled cells and remark that in their patient the symptoms were as severe as in homozygous Hb S disease although the concentration of intracellular Hb S was much less

Electrophoresis of Hæmoglobin The hæmoglobin of the cases mentioned above consisted of a mixture of Hb S and Hb D Hb A was absent Data on the proportions of Hb-D to Hb S are awaited A small amount of Hb F was present in Smith and Conley s patient

Sickle Cell Hæmoglobin/Hæmoglobin E Disease (Hb S/Hb E Disease) One Gene for Hb S and One Gene for Hb E

Because of the marked differences in the geographical distribution of the two hæmoglobins (see Figs 98 and 106) the combination of Hb S with Hb E is not likely to occur often Askoy and Lehmann (1957b) have however reported an Eti Turk family in which this combination was found twice

A 70 year old woman and one of her sons had a mild hypochromic microcytic anæmia Some target cells were present but otherwise the blood films were not remarkable Both subjects had slightly raised reticulocyte counts and serum bilirubin levels Their erythrocytes sickled *in vitro* and their hæmoglobin was found to consist of approximately 40 % Hb E and 60 % Hb S

the two sets of data and the authors pointed out that it may not be possible always to separate the two disorders on the basis of routine clinical and laboratory findings

The leucocyte count is usually within the normal range but in crises a leucocytosis is the rule Smith and Conley (1954) recorded a count of 59 000 per cu mm during a crisis in one of their pregnant patients

Electrophoresis of Hæmoglobin This reveals a rather constant pattern About 50–67% of the hæmoglobin is Hb C the remainder is Hb S except perhaps for a small amount of Hb F (Zuelzer Neel and Robinson 1956) Went and MacIver (1958b) recorded 0 7–8% of Hb F in their large series of patients

Sickle Cell Hæmoglobin/Hæmoglobin D Disease (Hb S/Hb D Disease) One Gene for Hb S and One Gene for Hb D

Only a very few families in which Hb S/Hb D disease has occurred have been reported (Itano 1951 Dacie 1954 Sturgeon Itano and Bergren 1955 Stewart and MacIver 1956 Smith and Conley 1959) The clinical syndrome is less serious than that of homozygous Hb S disease

The family investigated by Itano (1951) and subsequently reported in more detail by Sturgeon Itano and Bergren (1955) had been previously reported by Cooke and Mack (1934) as an example of sickle cell anæmia in a white American family

The patients consisted of a brother and sister aged 21 and 23 They were born of parents of Irish English and American Indian ancestry and Irish and English ancestry respectively The mother had the Hb D trait and the father Hb S trait Both children had a mild hæmolytic anæmia the brother suffered from repeated attacks of limb and abdominal pain but his sister who was only slightly less anæmic had few complaints Both had a similar blood picture there were *moderate numbers of partially sickled cells in the circulation* a moderate degree of anisocytosis and a small number of target cells The MCV was 113–118 cu μ Both blood samples sickled slowly *in vitro* The reticulocyte count was 7 2–8 1 % The brother s spleen had been removed in infancy his sister s was not palpable

Dacie (1954) reported the second family in which Hb S/Hb D disease occurred The propositions was a white girl 9 years of age who had been in hospital twice previously for pyrexia of unexplained origin She was moderately anæmic hæmoglobin 8 4 g per 100 ml and 2 000 000 erythrocytes per cu mm There were 9% of reticulocytes The MCV was 100 cu μ and the MCHC 32% Osmotic fragility was decreased

Blood films showed marked anisocytosis and anisochromasia and oat and sickled shaped cells were present (Fig 103) The child s father

reasons which are quite obscure became strongly established there and not elsewhere (Allison 1956a) In America Hb C is considerably less common than in Ghana most surveys reporting an incidence of 2–3% for the Hb C trait (Smith and Conley 1953 Schneider 1954 1956) On this basis one in about 6 000 negroes would be expected to be homozygous for Hb C

Although clearly a negro characteristic a small number of cases of Hb C trait or disease have been reported in other racial groups *e g* in a white family of Dutch and German origin (Huisman van der Schaaf and van der Sar 1955) in Italians (Erlandson Smith and Schulman 1956) and in white South Africans of Dutch parentage (Lewis Anderson and Baskind 1957) In Algeria Hb-C appears to be the most common type of abnormal hæmoglobin (Cabannes *et al* 1956)

Although not nearly as lethal as Hb S when homozygous those who are homozygous for Hb-C are probably at some disadvantage compared with normal individuals If so the same problems arise as with Hb-S how is it that the gene has been maintained at a high concentration in certain populations and have those who are heterozygous for Hb-C and Hb A some advantage over other genotypes? No satisfactory solution of these problems has as yet been put forward (Allison 1956a Edington 1959)

Inheritance of Hæmoglobin C The inheritance of Hb C is exactly the same as that of Hb S *i e* the gene is a non sex linked Mendelian dominant It appears to have a high degree of penetrance (Zuelzer Neel and Robinson 1956) All the evidence points to it being an allele of Hb A and Hb S

Clinical Features Associated with the Presence of *Hæmoglobin C*

Hæmoglobin C Trait (Hb A/Hb C) It is generally agreed that the presence of a single gene for Hb C practically never gives rise to any clinical symptoms Smith and Krevans (1959) nevertheless in a review of 240 cases observed one example of unexplained hæmaturia and the dental X rays of several other patients showed spotty bone formation

Hæmoglobin C Disease (Hb C/Hb C) (Homozygous Hæmoglobin C Disease) Spaet Alway and Ward (1953) were the first to describe a patient homozygous for Hb C Other case reports and records soon followed and the clinical syndrome is now well recognized Most of the pateints have been studied in America (Levin *et al* 1953 Ranney Larson and McCormack 1953 Schneider 1954 Terry Motulsky and Rath 1954 Singer Chapman *et al* 1954 Hartz and Schwartz 1955 etc) The majority have been discovered in surveys of the incidence of abnormal

HÆMOGLOBIN C

Hæmoglobin C was first described by Itano and Neel (1950) as ' hemoglobin III" the first clinical report of its presence in American negroes being published by Kaplan Zuelzer and Neel in 1951 There is now a large literature As already mentioned Hb C is an allele of Hb A and Hb S (Ranney 1954) and its combination with Hb S in a single individual leads to a mild form of sickle cell anæmia A single gene for Hb C has no clinical effect although the effect of its presence can be recognized in the

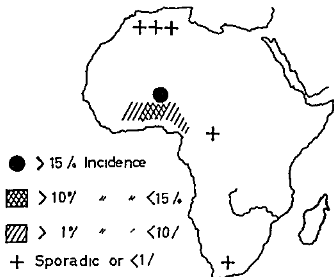


FIG 104 Distribution of Hb C in Africa Redrawn from Lehmann (1959b)

laboratory (Hb C trait) but two genes together give rise to a rather characteristic mild hæmolytic syndrome (Hb C disease) Hb C does not give rise to sickling of the erythrocytes

Geographical Distribution of Hæmoglobin C Edington and Lehmann (1954a) were the first to report the presence of Hb C outside the United States In a survey of blood samples from 200 Africans in Ghana an incidence of 12% was found and it is now clear that the main focus for Hb C is in West Africa (Allison 1956a c Neel *et al* 1956) (Fig 104) The highest frequency so far reported (20%) is in the Northern Territories of Ghana (Neel 1957) Presumably the gene arose there and for

seldom less than 50% (Fig 107 p 284) The erythrocytes sometimes fold over in one axis so as to produce boat shaped cells (Terry Motulsky and Rath 1954) or folded cells (Smith and Krevans 1959) This is probably the result of their extreme thinness

Another interesting phenomenon is the occurrence of crystals of hæmoglobin within the erythrocytes in Hb C disease This was first reported by Diggs and co workers (1954) in an Italian youth who had undergone splenectomy 2 years previously About 2% of the erythrocytes contained single crystals 6–10 μ in length and 2–3 μ in width The crystals appeared to be six sided and had blunt or pointed ends A further instance of the same phenomenon was reported by Wheby Thorup and Leavell (1956) also in a splenectomized patient Rod shaped erythrocytes (?) containing crystals were seen in small numbers in the peripheral blood of five of Smith and Krevans s (1959) patients and in large numbers in the blood of one patient after splenectomy

The reason for the development of intra-erythrocytic crystals and their relation to splenectomy are obscure No crystals could be found in smears of spleen pulp in Wheby Thorup and Leavell s case and it does not seem likely therefore that the spleen before removal had been actively filtering off cells containing crystals

Krauss and Diggs (1956) observed that intra-erythrocytic crystals developed when citrated blood from patients with Hb S/Hb C disease or Hb C/thalassæmia in whom the concentration of Hb C exceeded 58% was allowed to dry slowly *in vitro* Crystallization of normal blood did not occur nor of blood from subjects with Hb C trait in whom the content of Hb-C was less than 44 Ager and Lehmann (1957c) however in a similar study showed that several different types of hæmoglobin would undergo crystallization when kept *in vitro* in a medium of increasing osmolarity as might be produced by slow drying Hb C did not crystallize more readily than did the others

The reticulocyte count is typically slightly raised but may be normal Itano Bergren and Sturgeon (1956) reported counts up to 7% and Smith and Krevans (1959) stated that the reticulocyte counts exceeded 1 5% in twenty out of 32 patients and were greater than 6% in five of them

Osmotic fragility is definitely diminished Lewis Anderson and Baskind (1957) studied the effect on osmotic fragility of the previous incubation of the blood for 24 hours at 37 C Blood samples from three different patients showed increased resistance compared with normal blood a sample of Hb C trait blood gave *an intermediate result* The rate of *autohæmolysis* measured at 48 hours was less than normal but the addition of glucose failed to diminish the hæmolysis as it does with normal blood

hæmoglobins or as the result of the patients entering hospital for some reason other than anæmia They are nevertheless usually mildly anæmic and may complain of intermittent mild jaundice or abdominal discomfort Joint pains have occasionally been com plained of Leg ulcers or clinical signs of thrombotic lesions are not usually found nor is hæmaturia although this has been reported (Lewis Anderson and Baskind 1957) X ray studies of the skeleton have revealed nothing unusual The spleen is usually palpable but not grossly enlarged

Hb C disease seems to be compatible with a normal life expectancy and Ransone and Lange (1957) referred to a patient whose hæmoglobinopathy was first diagnosed when he was 79 this patient also had gout Crises similar to those of Hb S disease do not seem to be experienced

Smith and Krevans (1959) have recently reviewed the clinical manifestations of 35 cases of Hb C disease including nine personally studied patients Their symptoms were very variable but they included abdominal pain (nine of the 35 patients) joint pains headache hæmorrhagic phenomena and jaundice None of the patients complained of their eyes The spleen was palpable in 34 of the 35 patients There were nine uncomplicated pregnancies but one patient developed a superimposed severe megaloblastic anæmia

Hæmatological Findings

Hæmoglobin C Trait There is no anæmia However the excess of target cells in the blood film is characteristic although not diagnostic The appearances are not uniform some patients films showing no target cells others having as many as 40% (Kaplan Zuelzer and Neel 1953 Smith and Conley 1953 Watson 1956) There is typically no undue anisocytosis or poikilocytosis and the corpuscular constants are normal (Itano Bergren and Sturgeon 1956) Erythrocyte osmotic fragility is usually decreased Serum bilirubin levels are normal and survival studies indicate that the life span of the erythrocytes is probably normal There is no increase in Hb F (Watson 1956)

Hæmoglobin C Disease There is a variable degree of anæmia but the hæmoglobin is usually above 9 g per 100 ml it may be normal (see p 266) The erythrocytes are normocytic or slightly microcytic and there is usually only slight anisocytosis and poikilocytosis A few microspherocytes may be present Target cells are numerous and although this is a variable feature in some patients almost all the cells are affected the percentage is

Combinations of Hæmoglobin C with other Abnormal Hæmoglobins or with Thalassæmia

Hb S/Hb C disease has already been discussed (p 268) Other combinations except Hb C/thalassæmia are not well authenticated and seem likely to be very rare

Hb C/thalassæmia Singer Kraus and co workers (1954) described two members of the same negro family in which this diagnosis was made and Zuelzer and Kaplan (1954) reported a further patient also a negro Erlandson Smith and Schulman (1956) subsequently described the same syndrome in two white siblings of Italian origin Smith and Krevans (1959) have described a further case

The patients so far described have been mildly or moderately anæmic with relatively high erythrocyte counts raised reticulocyte counts (2–6 8%) and marked microcytosis (Itano Bergren and Sturgeon 1956) (Table 12 p 266) Stained films show marked pleomorphism of the erythrocytes with numerous target cells (30–60%) but also some microspherocytes (about 25% in Smith and Krevans s patient) as well as markedly hypochromic poikilocytes and cell fragments An increased span of osmotic fragility has been reported e g in Zuelzer and Kaplan s case 0 66–0 12% NaCl

Electrophoresis of Hæmoglobin Singer Kraus and co workers (1954) reported that the hæmoglobin in both of their patients consisted of about 75% of Hb C the remainder being Hb A with a little Hb F (2 4–2 7%) The hæmoglobin of Zuelzer and Kaplan s (1954) patient contained on the other hand far less Hb-C (29%) the remainder was Hb A and there was less than 2% of Hb F The two children reported by Erlandson Smith and Schulman (1956) were remarkable for the high concentration of Hb C 90% and 93% respectively only small amounts of Hb F were present (1 4 and 0 4%) Smith and Krevans s (1959) patient had 15% of Hb A and 2% of Hb F the remainder was Hb-C As with Hb S therefore the thalassæmia gene varies markedly from family to family in its ability to interact with Hb C in patients heterozygous for thalassæmia and Hb C

HÆMOGLOBIN D

Properties and Geographical Distribution As already mentioned Hb D was first described by Itano in 1951 Three samples of blood from members of a white American family suspected of having sickle cell anæmia were found to contain a

Leucocyte and platelet counts have usually been reported as normal Singer Chapman and their co workers (1954) however *mentioned* that one of their patients had thrombocytopenia (? hypersplenism) More recently Smith and Krevans (1959) stated that eight patients had been recorded as having thrombocytopenia

The serum bilirubin is normal or slightly raised and survival studies indicate that the mean life span of the erythrocytes is moderately reduced (Singer Chapman *et al* 1954 Terry Motulsky and Rath 1954 Thomas Motulsky and Walters 1955 Lange and Hagen 1955 Ransone and Lange 1957) Ferrokinetic studies *suggest* that the ability of the bone marrow to compensate *for* the increased hæmolysis may be less than normal (Thomas Motulsky and Walters 1955 Jensen Schoefield and Agner 1957) The reason for this is unknown Bone marrow biopsy shows normoblastic hyperplasia Megaloblastic erythropoiesis seems to have been reported only by Smith and Krevans (1959) in a patient who was pregnant

Electrophoresis of Hæmoglobin *Hb C Trait* About 25–39% of the hæmoglobin present is Hb C the remainder Hb A (Schneider *1954 Zuelzer Neel and Robinson 1956) Smith and Krevans* (1959) give the percentage of Hb C as 33% Hb F is not present in increased amounts

Hb C Disease 100% or almost 100% of the hæmoglobin is Hb C Hb A is absent but small amounts of Hb F may be present (Lange and Hagen (1955) 3 7% of Hb F Thomas Motulsky and Walters (1955) 2 4% of Hb F Watson (1956))

Thomas Motulsky and Walters (1955) studied an infant born of a mother who was homozygous for Hb C Hb C could not be identified at birth but it was definitely present 4 months later (*cf* Hb S which is present at birth although in relatively low concentration)

Pathology of Hæmoglobin C Disease Little information is available There are however reports on the histology of several spleens removed at operation (*Diggs et al 1954 Wheby Thorup* and Leavell 1956 Smith and Krevans 1959) or necropsy (Jensen Schoefield and Agner 1957) Increased congestion of sinuses and spleen pulp was reported and thickening of the capsule trabeculæ and inter sinusoidal reticulum Jensen Schoefield and Agner (1957) described the presence of multiple ante mortem thrombi within the pulmonary arteries in one case The relationship if any between these thrombi and the presence of Hb C is uncertain

out on negroes in the Mid Western United States Four examples of Hb D heterozygotes were found out of 1 000 blood samples examined Later Chernoff (1958) reported studies carried out on eleven heterozygotes Of the families in which Hb D was discovered, one was white the others negro In four of the negro families American Indian blood was said to be blended The limited family studies that have been carried out suggest that Hb D is an allele of Hb-A and Hb S (Chernoff 1958)

Hb-D is thus rarely found but it does not seem to be as rare as was first thought Further information as to its incidence and distribution is awaited As already mentioned chemical differences between Hb D and Hb S have been demonstrated It should be added that the same type of study has shown that at least three types of Hb D exist This fact may explain at least in part the rather erratic distribution of Hb D which has been reported up till now

Hæmoglobin D Trait (Hb A/Hb D) All authors agree that those who are heterozygous for Hb-D and Hb-A are not affected in any clinically discernible way The blood picture too is normal (Dacie 1954 Chernoff 1958) Electrophoretic studies reveal that rather less than 50% of the hæmoglobin present is Hb D the remainder is Hb A Hb F is not present in significant amounts

Hæmoglobin D Disease (Hb D/Hb D) (Homozygous Hæmoglobin D Disease) Only two reports of probable cases are yet available Bird and Lehmann (1956) mentioned a 25 year old Sikh in whom Hb D was the only hæmoglobin present This man was symptom free and there were no abnormal clinical signs He had however a slight anæmia and a microcytic erythrocytosis hæmoglobin 12 8 g per 100 ml erythrocytes 7 100 000 per cu mm MCV 63 cu μ MCHC 28% Numerous target cells were present and erythrocyte osmotic resistance was increased

Chernoff (1958) described a very similar case His patient was a 40 year old negress who had been diagnosed as being anæmic when aged 26 She had however no major symptoms and there were no abnormal physical signs There were 12–13 g of hæmoglobin per 100 ml and the erythrocyte count was 5 5–6 5 millions per cu mm The MCV was 67 cu μ and the MCHC 35% The reticulocyte count was normal There were 50–80% of target cells and the osmotic fragility curve showed a symmetrical shift towards increased resistance A ^{51}Cr survival study indicated a slight reduction in the erythrocyte life span

Based on these two reports Hb D disease would seem to produce a hæmolytic syndrome even milder than Hb C disease and wholly innocuous compared with that of Hb S disease

hæmoglobin component which had a mobility on moving boundary or paper electrophoresis identical with that of Hb S However the erythrocytes did not sickle and the solubility of the abnormal hæmoglobin when reduced was found to be far greater than that of Hb S The percentage of abnormal hæmoglobin in the samples ranged from 35 to 49%

Subsequent studies have confirmed that the solubility of Hb D is in fact approximately normal and that Hb D although superficially resembling Hb S is in reality quite distinct from it

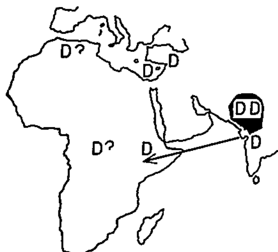


FIG 105 Distribution of Hb D (The Hb D in East Africa was found in Gujerati immigrants) Redrawn from Lehmann (1959b)

It can apparently be separated from Hb S by electrophoresis in agar gel (Smith and Conley 1959) and it has proved possible too to demonstrate chemical differences between the hæmoglobins (see p 295)

Hb D is now known to have a rather wide distribution it is apparently not confined to any one racial group (Fig 105) Thus it has been observed in India (Bird and Mourant 1955) in Algeria (Cabannes Sendra and Dalaut 1955a) in further Sikhs and Punjabi Hindus in India (Bird and Lehmann 1956) in Gujerati Indians domiciled in Uganda (Jacob Lehmann and Raper 1956) in Turkish and Persian families (Aksoy and Lehmann 1956 Hynes and Lehmann 1956) in the Belgian Congo (Vandepitte and Catrysse 1957) and in a Filipino family (Vella 1958a)

Chernoff (1956) published the results of an extensive survey carried

Erythrocyte osmotic fragility is usually slightly diminished (Chernoff *et al* 1956 Lie Injo Luan Eng and Ciok 1957)

According to Lie Injo Luan Eng (1956) Hb E is not present at birth it can however be detected in infants of more than 2 months of age In adults more than 20% but less than 50% of the hæmoglobin is Hb E The remainder is Hb A Hb F is not increased in amount

Hæmoglobin E Disease (Hb E/Hb E) (Homozygous Hæmoglobin E Disease) The syndrome associated with the presence of two genes for Hb E has by now been quite fully

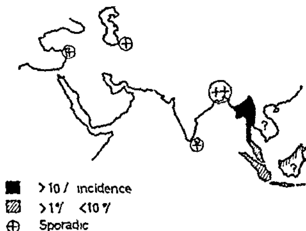


FIG 106 Distribution of Hb-E in the Old World Redrawn from Lehmann (1959b)

described (Chernoff *et al* 1956 Lehmann Story and Thein 1956 Lie Injo Luan Eng 1956 Na Nakorn and Minnich 1957) On the whole there is little disability and anæmia if present at all is only slight Referring to cases diagnosed in Cambodia (where the incidence of Hb E is apparently at its highest) Brumpt and co workers (1958) described the homozygous state not as a disease but as a terrain hématique fragile ' on which malnutrition parasites and above all thalassæmia lead to severe anæmia The six patients originally described by Chernoff and his colleagues (1956) were however noted to be of small stature and some of them gave a history of tiredness and arthralgia and possible jaundice The spleen was palpable in one and the liver in another Typically however the spleen is impalpable

Electrophoresis of Hæmoglobin In the two cases reported 100% of the hæmoglobin was Hb D Hb F was not present in appreciable amounts

Combinations of Hæmoglobin D with other Abnormal Hæmoglobins and with Thalassæmia

Hb S/Hb D disease has already been described (p 270)

Hb D/thalassæmia A possible example has been reported by Hynes and Lehmann (1956) in a Persian girl She had a mild anæmia with microcytosis (MCV 65 cu μ) and target cell formation (The blood picture was similar to the examples of Hb D disease quoted above) On electrophoresis her hæmoglobin was found to consist almost entirely of Hb D Hb A was absent but 4 2–4 9% of Hb F was present

The patient s father formed Hb A only (and presented no obvious signs of thalassæmia) the patient s mother could not be examined Hb D disease was an impossible diagnosis in view of the fact that the father did not carry the Hb D trait Hb D/thalassæmia seemed to be a reasonable alternative

HÆMOGLOBIN E

A fourth abnormal hæmoglobin (Hb E) was described by Itano Bergren and Sturgeon (1954) in an American family of partly Indian origin and by Chernoff Minnich and Chongchareonsuk (1954) in the blood of eight Thai subjects It was next identified in Ceylon (Graff *et al* 1954)

Since these early reports the abnormal hæmoglobin has been found to be widely spread in the Far East (Fig 106) It is present in its highest concentration in Burma (Lehmann Story and Thein 1956) Thailand (Na Nakorn Minnich and Chernoff 1956 Na Nakorn 1959) and Cambodia (Brumpt *et al* 1958) but it is also widespread in Malaya (Lehmann and Singh 1956) and Indonesia (Lie Injo Luan Eng 1955 1956 1959 Lie Injo Luan Eng and Giok 1957) and has been reported from the Philippines (Stransky 1958) India (Chatterjea 1959) and Turkey (Aksoy 1959) It is not apparently found in the Chinese (Na Nakorn Minnich and Chernoff 1956) There is every reason to believe that Hb E is an allele of Hb A The clinical and hæmatological results of its presence are remarkably similar to those produced by Hb C (Zuelzer Neel and Robinson 1956)

Hæmoglobin E Trait (Hb A/Hb E) Heterozygotes of Hb E and Hb A do not suffer any clinical disability and there is no anæmia However the erythrocytes tend to be microcytic although normochromic and a few target cells may be present In addition occasional cells may show fine basophilic stippling

children presented with splenomegaly pallor and intermittent jaundice Some had crises characterized by pyrexia and darkening of the urine Growth and sexual development are usually retarded and the abdomen eventually becomes enormously enlarged This is mainly the result of the splenomegaly but the liver may be enlarged also Bone joint and muscle pains are not complained of Ulcers of the leg occur but are uncommon

Blood Picture This closely resembles that of thalassæmia major (see p 266) The hæmoglobin level is usually less than 7 g per 100 ml and transfusions may be essential Stained films show small hypochromic erythrocytes and marked anisocytosis and poikilocytosis and usually from 5 to 25% of target cells (*cf* homozygous Hb-E disease in which the cells are much more uniform) A moderate degree of polychromasia and a few spherocytes (6–8%) are usually present The reticulocyte count is slightly raised (4–6%) the MCV usually ranges from 65 to 75 cu μ and the MCHC from 27 to 29% (Chernoff *et al* 1956) Erythroblasts are not uncommon and if splenectomy has been carried out they usually outnumber the leucocytes

The *erythrocyte osmotic fragility* is markedly diminished but there may be a small tail of fragile cells (*cf* thalassæmia major p 267) There is thus a much wider span of fragility than in homozygous Hb E disease After incubation the osmotic resistance increases still further on the whole although a small percentage of cells then undergo lysis in 0 85% NaCl (Punt and van Gool 1957)

The serum bilirubin concentration is raised and survival studies have demonstrated clearly that the erythrocyte life span is diminished The bone marrow is hyperplastic due to increased erythropoiesis

The majority of the patients reported by Chernoff and his co workers were severely affected and needed repeated transfusions Splenectomy which had been carried out in some of the children appeared to have been of some slight value

Hæmoglobin In Hb E/thalassæmia this has been reported as consisting of Hb E and from 55 to 85% of Hb F (Chernoff *et al* 1956) Hb A may apparently be present also in some cases (Lie Injo Luan Eng and Giok 1957)

Homozygous Hb E Disease plus Thalassæmia Trait

As there is reason to believe that the genes for thalassæmia and those for abnormal hæmoglobins such as Hb-S Hb-C and Hb-E are not alleles it is theoretically possible for the gene for thalassæmia to be present in a subject carrying two genes for an abnormal hæmoglobin This is only likely to occur where the hæmoglobinopathy and thalassæmia

Blood Picture This is definitely abnormal despite the absence as a rule of a significant degree of anæmia (see p 266) Stained films show that the erythrocytes are microcytes or normocytes Target cells are numerous (usually 25–75%) but anisocytosis poikilocytosis and polychromasia are not as a rule conspicuous According to Chernoff and his colleagues (1956) the MCV ranges from 65 to 72 cu μ and the MCHC is normal or only just subnormal (29–34%) Erythrocyte osmotic fragility is markedly diminished but the curve is symmetrical and not unduly sloped (Chernoff *et al* 1956) The reticulocyte count has usually been reported as normal In association with the microcytosis the erythrocyte count is often greater than normal and Lehmann Story and Thein (1956) reported a count of more than 8 000 000 per cu mm

The bone marrow is slightly hyperplastic and it seems possible that there is a minimal degree of excess hæmolysis Except however for one case (Na Nakorn and Minnich 1957) erythrocyte survival studies do not seem to have been carried out as yet

Hæmoglobin This consists of Hb E with small amounts of Hb F (up to 6 4% (Chernoff *et al* 1956)) Hb A is absent

Combinations of Hæmoglobin E with other Hæmoglobins and with Thalassæmia

The rare incidence of Hb S/Hb E disease has already been referred to (p 271) Hb E/thalassæmia is however an important and not infrequent cause of ill health in the Far East

Hæmoglobin E/thalassæmia (Hb E Trait plus Thalassæmia Trait) The first cases of this condition were described as examples of Mediterranean anæmia in Thailand by Minnich Na Nakorn Chongchareonsuk and Kochaseni (1954) On reinvestigation five of the patients were found to have Hb E/thalassæmia (Chernoff Minnich and Chongchareonsuk 1954 Chernoff *et al* 1956) Other cases (in Asiatic racial groups other than the Thais) have since been described by Sturgeon Itano and Bergren (1955) Lie Injo Luan Eng Mursadik Lioe and Odang (1956) Punt and van Gool (1957) and Nagaratnam and colleagues (1958)

Hb E/thalassæmia is a syndrome very similar to Cooley s anæmia (thalassæmia major) although somewhat less severe Chernoff and co workers (1956) have described the largest series of patients The symptoms vary widely in severity The disorder is usually discernible in the first year of life and in only four of the 32 patients investigated by Chernoff and co workers were major symptoms postponed until after the 10th year Most of these

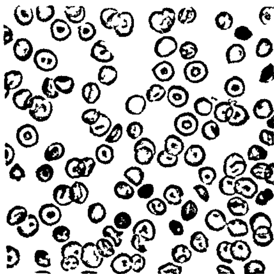


FIG 107 Photomicrograph of a blood film of a patient with Hb C disease. Numerous target cells are present and occasional folded cells, also a few spherocytes ×700

occur commonly side by side as in Thailand In their latest paper Na Nakorn and Minnich (1957) discuss the syndrome of homozygous Hb E disease and its distinction from Hb E/thalassæmia They studied a single family of ten members the mother was homozygous for Hb E and the father and three children were either heterozygous Hb E/ thalassæmia or homozygous Hb E/thalassæmia Unfortunately the two genetic combinations if indeed they were present side by side in this family did not seem to be associated with clearly distinguishable syndromes

HÆMOGLOBIN G

A hitherto unknown type of hæmoglobin was discovered by Edington and Lehmann (1954b) in the blood of a West African negro living in Ghana This hæmoglobin which was named Hb G migrated on paper electrophoresis slightly faster than Hb S but slower than Hb A It occupied approximately the same position as Hb F but unlike Hb F it was not abnormally resistant to alkali denaturation

A fuller report on the properties of Hb G was given by Edington Lehmann and Schneider (1955) It had been found possible to investigate the family of the propositus the mother had Hb A only and the father Hb G only being apparently homozygous of eleven offspring said to be legitimate ten including the propositus were heterozygotes for Hb G and one had Hb S trait Neither the heterozygotes for Hb G nor the presumed homozygote had any clinical symptoms attributable to the presence of the hæmoglobin their blood pictures were normal and they were not anæmic The blood did not sickle and Hb F was not present

Hæmoglobin G is one of the least common of the abnormal hæmoglobins It seems to have been next detected in an American family of Italian origin (Schwartz and Spaet 1955 Schwartz *et al* 1957) This family proved to be of great interest for in it Hb G Hb S and thalassæmia all occur together It is the study of this family that has led Schwartz and his co workers (1957) and Neel (1958) to conclude that Hb G is not an allele of Hb A and Hb S etc but like thalassæmia is controlled by a gene at a separate locus Study of this family also led the authors to conclude that the absence of Hb A in individual heterozygous for two hæmoglobin genes does not provide critical evidence that the genes are alleles

Schwartz and his co workers concluded as had Edington Lehmann and Schneider (1955) that there was no evidence that Hb G was clinically important either by itself or in combination with Hb S or thalassæmia in particular the patients with thalassæmia plus Hb G did not seem to be worse off than those with thalassæmia alone The latter observations are remarkable they place Hb G into a different category from Hb C or Hb D which in combination with Hb S lead to an active hæmolytic syndrome Schwartz and his co workers pointed out that in their patient who was heterozygous for both Hb S and Hb G the two hæmoglobins were present in the ratio 40 60 and that the relatively

low proportion of Hb S was probably the factor responsible for the lack of serious symptoms

A further example of Hb G this time in a Chinese woman has been recently reported by Vella Ager and Lehmann (1958)

'FAST MOVING HÆMOGLOBINS HÆMOGLOBINS H I J K ETC

The abnormal hæmoglobins which have been described in the preceding pages all migrate in an alkaline medium on moving boundary or paper electrophoresis more slowly than Hb A The first description of a new' hæmoglobin which moved faster than Hb A by Rigas Koler and Osgood in 1955 was therefore of unusual interest Now it is known that several distinctive types of hæmoglobin have this property All seem to be rare and only Hb H appears so far to have any clinical significance

Hæmoglobin H This was the designation given by Rigas Koler and Osgood (1955) to the abnormal hæmoglobin which they detected in two members of a Chinese family in which thalassæmia also occurred About 30% of the hæmoglobin present was Hb H The abnormal hæmoglobin was particularly remarkable for although found in two siblings it could not be detected in either of their parents

Further descriptions of fast moving hæmoglobins soon followed Gouttas Fessas Tsevrenis and Xefteri (1955) described four members of a family in which thalassæmia occurred and also three sporadic cases in whom the blood contained a fast moving abnormal hæmoglobin They noted moreover a remarkable phenomenon when the erythrocytes were suspended in brilliant cresyl blue solution the hæmoglobin underwent denaturation and in about 35–90% of the cells multiple blue staining round granules appeared (Fig 108) Gouttas and co workers reported that the inclusions could not be seen in fresh preparations of blood by either conventional or phase contrast microscopy or after staining with May Grunwald Giemsa The presence of brilliant cresyl blue seemed to be essential The granules started to become visible after 10–15 minutes and were most obvious after about 2 hours incubation

In 1956 Rigas Koler and Osgood gave more details of the family in which they had first observed Hb H and of the properties of the abnormal hæmoglobin Granule formation in reticulocyte preparations similar to that reported by Gouttas and his co workers was observed Rigas Koler and Osgood in considering the genetics of Hb H concluded that the most likely



Fig 108 Denaturation of Hb H by brilliant cresyl blue dye Th round bodies of varying size represent the Hb H × 900

inclusions on incubation and the father's cells also produce a very few inclusions This led Hedenburg and his co-workers to suggest that the boys parents might be heterozygous for Hb-H and the boys themselves homozygous There appeared to be no positive evidence for thalassæmia trait in the family In particular the Hb-A_2 concentrations were normal

From the above accounts the main foci for Hb H thus seem to be in the Far East (Fig 109)



FIG 109 Distribution of Hb-H Redrawn from Lehmann (19 9b)

Hæmoglobin I

An abnormal hæmoglobin designated Hb I was found to be present in six members of a negro family investigated by Rucknagel Page and Jensen (19..) They were not anæmic and their blood pictures were normal About 20 % of their hæmoglobin was Hb I 80 % was Hb-A At first sight this hæmoglobin appeared to be Hb H (for the mobilities of the two hæmoglobins at pH 8 6 seemed to be identical at pH 6 5 however Hb-I (and Hb A) travel towards the cathode while Hb H moves towards the anode (Fig 115))

Further descriptions of Hb-I are awaited it clearly is a rarity

Hæmoglobin-J

Hæmoglobin-J was first identified in the blood of a negress and in thirteen members of her family by Thorup Itano Wheby and Leavell (19.6) Its presence was not associated with anæmia or an abnormal blood picture (At pH 8 6 the mobility of the hæmoglobin was intermediate between that of Hb A and Hb I) at pH 6 . it moved with Hb I towards the cathode A remarkable feature when compared with other heterozygotes of an abnormal hæmoglobin and Hb A was the finding that more than one half (about 60 /) of the hæmoglobin present was Hb-J

Hb-J has subsequently been identified in an Indonesian family by Huisman Noordhoek and da Costa (19.7) (the two patients had 35 % of

explanation for the absence of Hb H in both parents of the three affected siblings was that the gene for Hb H produces no demonstrable abnormality unless that for thalassæmia trait is present as well Motulsky (1956) who had observed Hb H in two mildly anæmic Filipinos came to the same conclusion i e that thalassæmia–hæmoglobin H disease" (Hb H/thalassæmia) was a variant of thalassæmia in which the thalassæmia gene activates the expression of Hb H and that Hb H by itself is not demonstrable This view has been generally accepted (but see below (Hedenburg et al 1958))

The largest series of patients so far reported with Hb H/thalassæmia seems to be that of Minnich and her co workers (1958) Twenty eight patients in all were described twenty two were pure Thais five were of mixed parentage and one was pure Chinese Four of these patients had been originally reported in 1954 as suffering from Mediterranean anæmia by Minnich Na Nakorn Chongchareonsuk and Kochaseni in this paper incidentally there is a good photograph of the characteristic inclusions (of denatured Hb H) which at that time were unexplained The later descriptions of Hb H/thalassæmia (Minnich et al 1958) suggest that the course and severity of the disorder does not differ *significantly from uncomplicated thalassæmia minor (see p 266)* The degree of anæmia is not correlated with the amount of Hb H present Some of the patients of Minnich and her co workers were submitted to splenectomy in most of them this seemed to be followed by some improvement The inclusions became more easily demonstrable and 5–10% of the erythrocytes developed large granules immediately on contact with the brilliant cresyl blue solution

Since these early descriptions further cases of Hb H/thalassæmia have been observed for example in Indonesia in a family of mixed Chinese and Indonesian ancestry (Lie Injo Luan Eng et al 1957) in Italy (Silvestroni and Bianco 1957) in a child of Italian origin (Wolff Michaels and Von Hofe 1958) in a Nepalese Gurkha woman (Brain and Vella 1958) in two Greek Cypriot families (Bingle Huehns and Prankerd 1958) and in a Chinese patient (Jim 1958) Fessas (1959a) gives further details of patients studied in Athens

Hæmoglobin H has also been observed in two brothers of Swedish origin (Hedenburg et al 1958) and in a Transjordan Arab who also had leukæmia (Dacie and White unpublished observations)

The account of Hedenburg and his co workers is particularly interesting Two small boys of Swedish origin presented with mild *anæmia (hæmoglobin 9 4–10 5 g per 100 ml)* 24–27% of their erythrocytes developed inclusion bodies on incubation with brilliant cresyl blue and from ~-13 5 / of Hb H was present The boys mother and two of her relatives had erythrocytes which produced a few

Further details of Hb-M are given by Gerald (1958) In an addendum to this paper he reports that more than one type of Hb-M exist (see also Gerald and George (1959))

Hæmoglobin N

As Liberian I this abnormal hæmoglobin was first discovered by Robinson and his co-workers in seven out of 920 blood samples tested in Liberia It was subsequently thought to be identical with Hb-J This is apparently not so and the label Hb-N originally reserved for Liberian II has therefore been bestowed on it (Ager and Lehmann 1958) Hæmoglobin N has also been reported from Portuguese Guinea (Trincão Almeido Franco and Nogueira, 1959)

Hæmoglobin O

This title has been given to the abnormal hæmoglobin found by Lie Injo Luan Eng and Sadono (1958) to be not uncommon in Sulawesi (Celebes) It was originally referred to as Buginese X On paper electrophoresis at pH 8 6 it migrates between Hb S and Hb-E Studies by column chromatography and in the Tiselius apparatus at pH 6 5 and pH 8 6 suggest that it is different from any previously described hæmoglobin

Hæmoglobin P

This letter has been reserved for the abnormal hæmoglobin provisionally named Galveston which was identified in a negress and her child by Schneider and Haggard (1957) should this be conclusively shown to be a distinct type On paper electrophoresis at pH 8 6 the Galveston hæmoglobin overlapped Hb G and resembled Hb-L However on moving boundary electrophoresis it formed a single sharp peak Further details are given by Schneider and Haggard (1958)

Hæmoglobin Q

This hæmoglobin has been found in association with Hb H in a Chinese man, and also in his mother in association with Hb-A (Vella *et al* 1958) The propositus presented with a hypochromic anæmia refractory to treatment the appearances suggesting Hb-H/thalassæmia However on electrophoresis on paper about 80 % of the hæmoglobin migrated at pH 8 6 between Hb-A and Hb S The remainder of the hæmoglobin was Hb H At pH 6 5 the new hæmoglobin separated from Hb H and moved to the cathode On ion-exchange chromatography the hæmoglobin separated from Hb G and moved between Hb-S and Hb L (Fig 116)

Other Hæmoglobins Newly Described as yet Unidentified or Unnamed

Three new types of fœtal hæmoglobin have been reported

(a) *The Fast Hæmoglobin of Fessas and Papaspyrou* (1957) This hæmoglobin was found in an infant it migrated between Hb H and Hb I on paper electrophoresis at pH 6 5 and between Hb-K and Hb J at pH 8 6 At birth 14% of the hæmoglobin was of the new type at 3 months only 4% Both parents had thalassæmia and it was suggested

Hb J) by Raper (1957) in a *Gujerati Indian* by McCabe Lange and Crosby (1957) in a young man with Fanconi s anæmia and splenic atrophy and by Lie Injo Luan Eng (1958) also in an Indonesian family Recently Sanghvi Sukumaran and Lehmann (1958) have reported the finding of Hb J in two Indian women one of whom was suspected of having thalassæmia minor also However her blood picture was similar to that of her husband (who also had thalassæmia minor) and there appeared *therefore to be no evidence of interaction between* the genes for Hb I and thalassæmia

Hæmoglobin K

Hæmoglobin K was first reported by Cabannes and Buhr (1955) as occurring in two Algerian families The hæmoglobin (at first referred to as Hb I) was found to migrate on electrophoresis at pH 8 6 between Hb A and Hb H It was next observed in two out of 900 Liberian subjects studied by Robinson and co workers (1956) who gave the hæmoglobin the temporary title of Liberian II

More recently it has been reported in an East Indian and his family (Ager and Lehmann 1957a) in a family of North Indian origin and in two pure Malay families by Vella (1958b) and in a further Indian family by Vella and Wells (1959)

On electrophoresis Hb K moves faster than Hb A at pH 8 6 but is barely separated from it it is slower than Hb J At pH 6 5 Hb K is attached to the rear of Hb A Further details of the incidence of Hb K *in Algeria and of laboratory findings are given by Cabannes and Buhr* (1958)

None of the subjects in whom Hb K has been found in combination with Hb A has been anæmic or has had an abnormal blood picture

More Recently Described Hæmoglobins

Hæmoglobin L

This hæmoglobin was identified in a Punjabi Hindu and his mother by Ager and Lehmann (1957b) At pH 8 5 the abnormal component moved between Hb S and Hb G and on column chromatography between Hb S and Hb C it formed about 28% of the total hæmoglobin The rate of denaturation by alkali and the solubility of the hæmoglobin appeared to be normal The propositus was not anæmic

Hæmoglobin M

The designation Hb M was used by Singer (1955) to describe an abnormal type of methæmoglobin first observed by Horlein and Weber (1948) in a family suffering from hereditary methæmoglobinæmia A further family of German origin residing in the United States who have a similar abnormality has recently been discovered (Gerald Cook and Diamond 1957) In the latter family it could be shown using starch electrophoresis that an abnormal oxyhæmoglobin as well as an abnormal methæmoglobin were present alongside normal oxyhæmoglobin The spectral absorption curve of the abnormal methæmoglobin was different from that of normal methæmoglobin there was no maximum at 630 mμ



Fig 110 I I otomicrograph of ckled erythrocytes A sealed preparat on of the blood of a patient with Hb-S disease Fully sickl d filamentou forms pred minate × 900



F g 111 Pl otom erograp l of ickl d erythrocytes from a patient w th Hb-S tra t A sim l r preparation to that shown in F g 110 Holly I af sickling i sl own × 900

that the new hæmoglobin was made manifest by the simultaneous presence of a gene for thalassæmia The hæmoglobin was *not* resistant to denaturation by alkali

(*b*) *Hæmoglobin Barts* This hæmoglobin was found in an infant 4 weeks old (Ager and Lehmann 1958) On paper electrophoresis at pH 8 6 it resembled Hb N but on chromatography it moved faster than Hb H Like Hb F but unlike the hæmoglobin of Fessas and Papaspyrou it was resistant to denaturation by alkali The infant and both parents may have had thalassæmia

(*c*) *Hæmoglobin Alexandra* This hæmoglobin was described by Fessas Mastrokalos and Fostiropoulos (1959) in the blood of a healthy infant At birth 18% of abnormal hæmoglobin was present and at 15 weeks 2% Its resistance to alkali was similar to that of Hb F On paper electrophoresis at pH 8 6 it migrated between Hb E and Hb S It could not be separated from Hb A on electrophoresis in agar

A hæmoglobin with properties similar to that of Alexandra has independently been isolated by Vella Ager and Lehmann (1959) from several cord blood samples obtained in Singapore

New Types of Adult Hæmoglobin

(1) *A Hæmoglobin in a Frenchman from Picardy* This occurred in a family harbouring thalassæmia (Andre *et al* 1958) About 50% of the hæmoglobin was abnormal In some respects the abnormal hæmoglobin resembled Hb E It was not however demonstrable in the blood of either parent of the propositus (*cf* Hb H)

(2) *Hæmoglobins Hopkins I and Hopkins II* (*Smith and Torbert 1958*) Each type of hæmoglobin was found once in 6 000 blood samples tested in Baltimore Both migrated faster at pH 8 6 than Hb A Hæmoglobin Hopkins II may be identical with Hb J and evidence was obtained that its gene was probably located on a different chromosome from that on which the Hb A S locus is situated

(3) *Hæmoglobin Sud Vietnam* (*Albahary et al 1958*) This so far unidentified hæmoglobin migrated on paper electrophoresis at pH 8 6 between Hb G and Hb A It was found in 20 blood samples from inhabitants of South Vietnam

(4) *Hæmoglobin Norfolk* (*Ager Lehmann and Vella 1958*) This hæmoglobin was the only abnormal one found in the course of examining the hæmoglobins of 2 550 Britons in Singapore The father and brother of the propositus who lived in Norfolk also were heterozygotes for the new hæmoglobin Their blood pictures were normal The hæmoglobin moved slightly faster than Hb J on paper electrophoresis at pH 8 6 The occurrence of this hæmoglobin is particularly interesting as it appears to be the first abnormal one found in a pure English family

DIFFERENTIATION OF THE ABNORMAL HÆMOGLOBINS

The original discovery by Pauling Itano Singer and Wells (1949) that the hæmoglobin in sickle cell disease was different from and could be separated from normal hæmoglobin was made using the technique of moving boundary electrophoresis in a Tiselius

apparatus This method has since been widely employed in later studies However the finding that good separation could be obtained by zone electrophoresis on paper strips (Spaet 1953 Schneider 1953 Smith and Conley 1953 Larson and Ranney 1953 Motulsky Paul and Durrum 1954 etc) proved to be an enormous stimulus to further work for it meant that preliminary studies at least of abnormal hæmoglobins could be undertaken in almost any clinical laboratory (Figs 112 and 113)

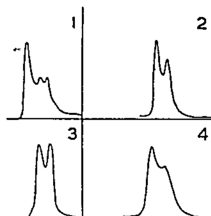


FIG 113 Human hæmoglobins separated by paper electrophoresis and recorded by scanning with a Laurence densitometer

1 Hb-A mixed with the hæmoglobin of a patient with Hb-S/Hb C disease Hb-A = large peak Hb S = left hand small peak Hb C = right hand small peak
2 Hb D trait Hb-A = large peak Hb D = small peak.
3 Hb S/Hb-C disease Hb S = left hand peak Hb C = right hand peak
4 Hb S trait Hb-A = large peak Hb S = small peak

The amounts of hæmoglobin present can be estimated approximately quantitatively by measuring the areas under the peaks (From White and Beaven (19 4))

It is beyond the scope of this book to review the technical factors which influence the results obtained by electrophoresis or to discuss in detail the other physical and chemical methods which have been used in the differentiation of the hæmoglobins Several excellent recent reviews are available *e g* those of Itano (1956 1957) Itano Bergren and Sturgeon (1956) Zuelzer Neel and Robinson (1956) Goldberg (1957) Jonxis and Huisman (1958)

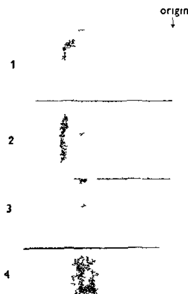


FIG 112 Results of paper strip electrophoresis of human hæmoglobin at pH 8 6

The arrow marks the place of origin 1 = Hb A ~ = Hb S trait (Hb A + Hb S) 3 = Hb S disease (Hb S only) 4 = Hb S/Hb C disease (Hb S + Hb C) (From Dacie (19ɔf))

studies resistance to denaturation by alkali chromatographic separation by an ion exchange resin (IRC 50 column) or on carboxymethylcellulose (Huisman Martis and Dozy 1958) and spectrographic measurements Other more specialized techniques that have been employed include sedimentation and diffusion studies amino acid and sulphydryl group analyses and oxygen equilibrium studies It has been stressed many times that it is

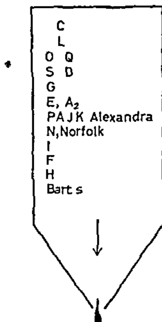


FIG 116 Sequence of separation of hæmoglobins in an Amberlite IRC-50 ion exchange column at pH 6 2
Hb P does not separate from Hb A but forms a tail (Based on a diagram kindly drawn for the author by Dr H Lehmann)

usually not possible to characterize an abnormal hæmoglobin as new or unique until it has been subjected to a whole battery of tests of which moving boundary and paper electrophoresis at acid and alkaline pH solubility measurements resistance to alkali and chromatographic separation are the most important

In the identification of an abnormal hæmoglobin suspected of being a new one it is absolutely essential to test it side by side with

Huisman (1959) and Beaven and Gratzer (1959) I shall however enumerate the more important methods that have been employed

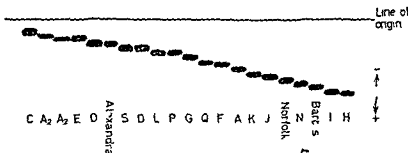


FIG 114 Mobilities on paper electrophoresis at pH 8 6 of carboxyhæmoglobins

Hb M moves like Hb A The three Hb D s move together Hb A_2 is a variant of Hb A_2 which segregates as an allele of Hb A_2 (Ceppellini Kunkel and Bunn (1958) quoted by Lehmann (1959a))

As referred to on p 295 (Hb-D comprises at least three hæmoglobins with identical electrophoretic chromatographic and solubility properties which are however different when examined by peptide analysis) (Benzer Ingram and Lehmann 1958) (Based on a diagram kindly drawn for the author by Dr H Lehmann)

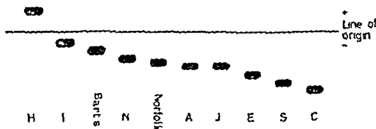


FIG 115 Mobilities on paper electrophoresis at pH 6 5 of carboxyhæmoglobins

Hb H is the only hæmoglobin to move to the anode Hb Norfolk barely separates from Hb A Hb N separates well from Hb A Hb-J does not separate from Hb A (Based on a diagram kindly drawn for the author by Dr H Lehmann)

The methods are moving boundary electrophoresis at various pH s usually at pH 8 6 in barbitone buffer or at pH 6 5 in cacodylate buffer zone electrophoresis at pH 8 6 or 6 5 on paper starch block or in agar gel (Robinson *et al* 1957) solubility

provides strong support for the concept that Hb A Hb S and Hb C are alleles

Further extensions of this work have recently been reported Benzer Ingram and Lehmann (1958) have differentiated three different types of Hb D all with chemical changes affecting different parts of the polypeptide chains of the hæmoglobin half molecule and Ingram (1959) reports that in Hb E lysine has been substituted for glutamic acid as in Hb C but this time in a different part of the molecule

The finding that Hb D consists of at least three hæmoglobins which have different chemical constitutions although having identical electrophoretic chromatographic and solubility properties is remarkable It suggests that hæmoglobins with apparently similar physico chemical properties discovered in widely separated parts of the world may also not be identical when subjected to critical chemical analysis (see Vella Ager and Lehmann 1958) The results of further studies can hardly fail to be of great interest

Erythrocyte Survival Studies in Sickle Cell Disease and Allied Disorders

Measurements of cell survival have demonstrated as might be anticipated from clinical data that the life span of the erythrocytes is considerably diminished in Hb S disease and diminished to a minor degree in Hb S/Hb-C disease etc Normal erythrocytes *transfused to patients with hæmoglobinopathies characteristically* survive normally

The first direct measurements of the life span *in vivo* of the erythrocytes in Hb S disease were reported by Singer Robin King and Jefferson (1948) and Callender Nickel Moore and Powell (1949) Using the Ashby technique marked differences in the survival of the transfused cell were observed a proportion of the cells disappeared rapidly from the circulation others were eliminated far more slowly Singer and Fisher (1952) correlated the difference in survival time with the relative amounts of Hb S and Hb F present and thought that the cells containing the greatest amount of Hb F survived the longest

Weinstein *and co workers (1954) studied the survival of the* erythrocytes of a small number of patients in their own circulations using ^{51}Cr As expected a major increase in cell destruction was found in Hb S disease (one patient gave a definitely two-component curve of elimination) and minor increases were found in Hb C disease and Hb S/ Hb C disease The results were normal in Hb S trait and Hb C trait James and Abbott (1955) reported the result of simultaneous [^{15}N]hæmin and [^{15}N] stercobilin studies in a patient with severe Hb S disease The erythrocyte half life was computed to be 11 days with a mean life span

well authenticated specimens of the hæmoglobins which it more superficially resembles Ager Lehmann and Vandepitte (1958) recommended hanging strip rather than horizontal paper electrophoresis when comparing hæmoglobins which migrate at about the same rate

Some of the properties of the various types of hæmoglobin that have been described earlier in this Chapter have been mentioned in the general description of each hæmoglobin Their relative mobilities on paper electrophoresis at pH 8 6 and 6 5 and on resin column chromatography are represented in Figs 114–116

Chemical Differences in the Abnormal Hæmoglobins

Almost as soon as Hb S was recognized as a distinct form of hæmoglobin it was realized that the difference between it and Hb A lay in the globin part of the molecule and not in the hæm (Pauling *et al* 1950) Subsequent work has been concerned with the exact elucidation of these differences The main interest has centred around the amino acid composition and number of amino acid residues per molecule and titrations for sulphdryl groups Major differences exist between Hb F and Hb A the former has an increased content of *iso*leucine as well as possibly altered proportions of other amino acids The differences between the abnormal hæmoglobins and Hb A have proved to be more subtle and less easy to demonstrate (see reviews of Itano (1956) Huisman (1958) and Beaven and Gratzer (1959))

The recent work of Ingram (1956 1957 1959) and Hunt and Ingram (1958) has pinpointed and explained the nature of these differences

The difference between Hb S and Hb A apparently lies in the substitution of a valine residue for a glutamic acid residue in one place in the 300 amino acids forming the polypeptide chain of a half molecule (Ingram 1957) This remarkable observation is especially significant in that it demonstrates the chemical consequences of a single gene mutation Analyses carried out on Hb C have demonstrated an analogous abnormality the same glutamic acid residue is replaced but this time by a lysine residue This change from an acidic to a basic amino acid involves the loss of two net changes per half molecule (twice as many as for Hb S) and explains the electrophoretic differences between Hb C Hb S and Hb A Hunt and Ingram (1958) concluded that the chemical alterations are the result of small alterations in the corresponding places on the gene controlling Hb A formation The fact that the chemical changes occur at the same place in the polypeptide chain

the mean ^{51}Cr half time was 15 7 days This report certainly emphasizes that the part played by the spleen in the production of anæmia in children with Hb-S disease is far from being negligible (see also p 308)

A ^{51}Cr erythrocyte survival curve carried out on a patient with Hb S disease is illustrated in Fig 117 The mean cell life span in this case was estimated to be about 9 days

References to other papers in which data on erythrocyte survival are given are to be found in the sections dealing with the individual hæmoglobinopathies

THE PATHOGENESIS OF SICKLE CELL DISEASE AND ALLIED CONDITIONS

All authors agree that the hæmolytic and thrombotic phenomena so characteristic of sickle cell disease are to a large extent the direct consequence of the sickling of the erythrocytes In the following discussion the process of sickling itself will therefore be considered first

The Sickling Phenomenon

As referred to in the introduction to this Chapter it was Emmel in 1917 who published the first description of the sickling of blood *in vitro* in sickle-cell disease The next advance was the recognition that the phenomenon occurred faster in active cases than in latent cases (Sydenstricker 1924)

Relationship between Oxygen Tension and Sickling In 1927 was published the classic paper of Hahn and Gillespie who were the first to relate sickling *in vitro* to a reduction in the partial pressure of oxygen They concluded that sickling was a reversible phenomenon which depended on whether the hæmoglobin was free or combined and that the discoid form of the cell was stable when the hæmoglobin was combined (with oxygen) and the distorted form stable when the hæmoglobin was free They added that the failure of corpuscles which have lost their hemoglobin to undergo sickle cell formation is consistent with our hypothesis relating the distortion to the hemoglobin Twenty two years were to pass before this hypothesis was proved to be correct

Hahn and Gillespie s conclusions received support from the in vivo studies of Scriver and Waugh (1930) The latter authors in a study of the effect of venous stasis on the number of sickled erythrocytes in blood withdrawn from below a tourniquet found that the number definitely increased if the partial pressure of oxygen was reduced below 40–45 mm Hg They concluded that anoxia after death was the cause of the large masses of sickled cells which might be seen *post mortem*

of 16 days They concluded that destruction was random and found no evidence of more than one population

Chernoff Rucknagel and Jim (1955) transfused blood from Hb S/Hb C disease patients into normal recipients with or without spleens Using the Ashby method they found that the transfused cells were completely eliminated in 50–60 days irrespective of whether or not the spleen had been removed These results underline the comparatively small role the spleen plays in the mechanism of hæmolysis Chaplin Keitel and Peterson (1956) also carried out some interesting experiments They transfused three patients with Hb S disease so as to raise the level of hæmoglobin to normal and followed the rate at which the percentage of sickled cells diminished in the circulation following the transfusion

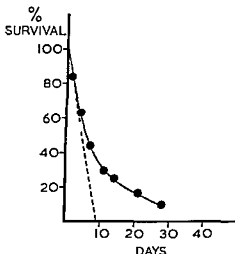


FIG 117 Survival of ^{51}Cr tagged erythrocytes of a patient with Hb S disease The mean cell life span is approximately 9 days

(at a time when the rate of erythrocyte regeneration was low) They concluded that their data indicated that some of the patients cells had a very short life span

The recent report by Sprague and Paterson (1958) on erythrocyte survival in Hb S disease and Hb S/Hb C disease and on the effect splenectomy has on survival seems to be the most extensive that has yet been published Seven children with Hb S disease and splenomegaly were studied six also after splenectomy the mean ^{51}Cr half time before operation was 4 0 days after operation it was 11 4 days (normal 30 ± 3 days) In seven older patients without splenomegaly (no splenectomy) the mean ^{51}Cr half time was 10 days while in four of five experiments in which blood from these patients was transfused to normal recipients (with spleens) the survival was considerably less (^{51}Cr half time 4 4 days compared with 9 2 days in the patients themselves) In seven patients with Hb S/Hb C disease (three with splenomegaly)

Demonstration of Sickling The change was originally observed in a fluid preparation of blood kept sealed beneath a cover slip Under these circumstances gradual deoxygenation takes place and the erythrocytes undergo a progressive distortion in a matter of minutes or hours Eventually in sickle cell disease *all the cells become changed to crescentic forms some with* associated spines or filaments (Fig 110)

A number of techniques have been suggested for bringing about sickling more rapidly by *hastening the deoxygenation of the blood* Sodium bisulphite and vitamin C (Daland and Castle 1948) or buffered isotonic sodium dithionite at pH 6 8 (Itano and Pauling 1949 Williams and Mackey 1949) or even a culture of *B subtilis* (Singer and Robin 1948) have been added to blood to bring this about Most workers seem to use sodium dithionite

Details of the sequence of changes were well described by Ponder (1945) In some cases the cells become less obviously sickle shaped and may assume instead a holly leaf appearance with multiple spines (Sherman 1940 Perosa *et al* 1957) (Fig 111) There is reason to believe that the formation of markedly distorted filamentous sickled forms is greater in Hb S disease than it is in the Hb-S trait (Diggs Ahmann and Bibb 1933–34 Neel 1951) Hb S/Hb C disease or Hb-S/thalassæmia The changes certainly occur more rapidly in Hb-S disease than in the trait

The findings on electron microscopy are summarized by Knights Clermont Sturrock and Schultz (1955) by Harris and co-workers (1956) and by Bessis Nomarski Thiery and Breton Gorius (1958) Bessis and his co-workers who studied sickled cells by a variety of optical methods concluded that the optical evidence was in favour of the hypothesis that gel formation rather than actual crystallization of the hæmoglobin took place within the sickled cell (see p 301)

Although sickling occurs at birth it takes place less readily in the blood of newborn infants than in later life (Watson Stahman and Bilello 1948 Scott Crawford and Jenkins 1948 Schneider and Haggard 1955 Shields *et al* 1958) For instance Watson and co-workers showed in a series of newborn negro infants that from 0 5 to 29 5 of their erythrocytes sickled compared with 84 to 100 sickling in the blood of the infants mothers One infant s blood was studied at frequent intervals it was found that the proportion of cells that would sickle increased from 6 at birth to 90 % at 4 months. It was considered possible that the large amount of fœtal hæmoglobin present at birth protects the cells in some way from the effects of a reduced oxygen tension It is certainly possible to show that the proportion of cells which sickle is inversely related to the amount of Hb-F present (Allison 1954b Shields *et al* 1958))

While it may be true that the presence of Hb-F may be less favourable for sickling than the presence of Hb A in the erythrocytes of carriers of the Hb-S trait possibly a more important factor is that little Hb-S is

The above mentioned early studies on the relationship between oxygen tension and sickling have been repeatedly confirmed and elaborated Sherman (1940) seems to have been the first to have distinguished between the behaviour of erythrocytes from subjects with Hb S trait or Hb S disease respectively He observed that in order for sickling to occur the atmospheric pressure had to be reduced far more in Hb S trait than in Hb S disease

Lange Minnich and Moore (1950 1951) carried out more elaborate studies They found working with oxygen-nitrogen mixtures with 10% of added carbon dioxide that Hb S disease erythrocytes sickled in 4–6 % oxygen while Hb S trait cells did not sickle until the percentage of oxygen was less than 2 % They also noticed that Hb S trait cells sickled at pH 6 9 or less when exposed to 2% oxygen while those of Hb S disease sickled at a pH of 7 –5 or less

Harris Brewster Ham and Castle (1956) carried out elaborate experiments in order to demonstrate the relationship between oxygen tension and sickling *in vitro* increase in blood viscosity and mechanical fragility They showed that in Hb S disease sickling could take place at physiological oxygen tensions *e g* at 40 mm Hg while in the trait an unphysiological tension of 10 mm or less was required They also found that oxygen tensions at which tactoid formation could be induced in solutions of Hb S were limited to the tensions which caused sickling of intact cells They concluded that a lowered pH increased sickling by facilitating deoxygenation of hæmoglobin

Griggs and Harris (1956) compared the findings in Hb S/Hb C disease and Hb S/thalassæmia with those in Hb S disease They found that while in Hb S disease (with 90–100% of Hb S) an oxygen tension of 60 mm Hg might produce sickling *in vitro* in Hb S/Hb C disease or Hb S/thalassæmia (with 50–80 % of Hb S) an oxygen tension of 40 mm Hg was required and in Hb S trait (with 24–48 / Hb S) a tension of less than 10 mm Hg They concluded that the most sensitive index of sickling was an increase in mechanical fragility (? due to the formation of intracellular small tactoids) then tactoid formation in hæmoglobin solutions and finally (as the least sensitive index) overt sickling and viscosity changes which ran parallel to each other

Allison (1956b) also studied the sickling process in detail He found that at a physiological pH sickling of Hb S disease erythrocytes took place at 40 mm Hg oxygen partial pressure when about 45% of the hæmoglobin was deoxygenated Individual cells varied in their sensitivity to sickling some not sickling until the pressure was reduced to 20 mm Hg or less Allison also observed that the cells from Hb S/Hb C and Hb S/thalassæmia heterozygotes reacted in a way intermediate between those of patients with Hb S disease or Hb S trait he concluded that sickling took at least 2 minutes to take place and that this fact had an important bearing on the genesis of symptoms *in vivo* Allison (1957b) studied the viscosity changes following deoxygenation of stroma free hæmoglobin solutions and concluded that Hb S formed mixed aggregates with Hb A or Hb C

In a more recent paper the workers at the Thorndyke Memorial Laboratory (Greenberg Kass and Castle 1957) showed that a positive correlation exists between the ease with which sickling and increases in blood viscosity take place on deoxygenation of blood and the actual concentration of Hb S in the erythrocytes

These earlier studies have been generally confirmed and the hypothesis that the hæmoglobin in sickled cells is in the form of tactoids of reduced hæmoglobin seems to have been generally accepted (Harris *et al* 1956 Allison 1957b) Harris and his

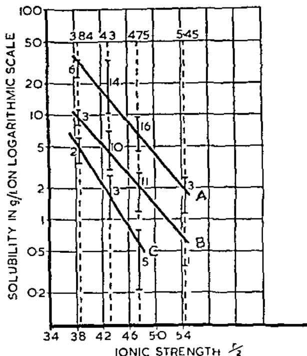


FIG 118 Solubility of reduced hæmoglobin in pH 6 ~ phosphate buffer

The regression lines are fitted to experimental values for hæmoglobin derived from normal adult blood (A) from Hb S trait blood (B) and Hb-S disease blood (C)
The range of values for each group at different ionic strengths is shown by the vertical bars The figures indicate the number of observations in each group (From White and Beaven 1954)

co workers pointed out that the slight loss of cell water which takes place when a cell sickles the appearances on electron microscopy and the results of X ray scattering studies fail to support the hypothesis of a crystalline structure for the sickled cell The optical studies of Bessis and his co workers (1958) also

present at birth According to Schneider and Haggard (1955) in infants with Hb S trait the amount present varies from 0 to 21% increasing to the adult level by about 4½–5 months

✓Leaving aside the age of the patient there are differences too in the ease with which erythrocytes of different ages sickle Watson (1948) found that whereas most reticulocytes sickled as readily as adult corpuscles the most immature ones sickled more slowly whilst mature normoblasts sickled more slowly still Watson also pointed out that the sickled cells seen in stained smears of air dried films of peripheral blood were almost invariably adult non reticulated corpuscles

Mechanism of Sickling The actual mechanism of sickling has been the subject of much research Sherman in 1940 was the first to observe that cells which underwent sickling became birefringent Pauling and his co workers (1949) suggested that under conditions of reduced oxygen tension the molecules of Hb S underwent a partial alignment within the cells and that elongation of the cells in one axis and distortion followed from this (Harris (1950) suggested that linkage of individual molecules led to the formation of long tactoids *(liquid crystalline masses)*)

Perutz and Mitchison (1950) showed that reduced Hb S was far less soluble than reduced Hb A Whereas the solubility of reduced Hb A was about one half of that of oxygenated Hb A the solubility of reduced Hb S was no more than one hundredth of that of oxygenated Hb S They suggested that the sickle shape and *birefringence of the distorted cells resulted from crystallization of* the hæmoglobin within the cell membranes

Further studies of the differences between Hb A and Hb S were carried out by Perutz Liquori and Eirich (1951) Using a slightly different technique they found that the solubility of reduced Hb S was about one tenth of that of reduced Hb A As a result of other experiments they concluded that the solubility of reduced Hb S was only one seventh of that required to keep the hæmoglobin in solution in the cell

The differences in solubility between Hb S and Hb A provide one method for the distinction between the Hb S trait and Hb S disease as the observed solubility seems to be a direct reflection of the proportion of Hb S present (Fig 118) Itano (1953a) found that the solubility of the hæmoglobin mixtures in the different types of sickle cell disease could be ranged in the following order (1) Hb S trait (most soluble) (2) Hb S/Hb C disease (3) Hb S/thalassæmia (4) Hb S disease (least soluble) Singer and Singer (1953) in studies in which they measured the minimum concentrations of Hb S which would undergo gelling when deoxygenated observed that the gelling point was modified by the presence of other types of hæmoglobin In Hb S trait for instance the presence of Hb A diminished the minimal amount of Hb S required for gelling and Hb C reduced this still further Singer and Singer made the point that sickling is caused not only by the presence of Hb S but also by its interaction with other hæmoglobins

tension has to be lowered to produce sickling appears to explain why the consequence to the patient of having Hb S trait are so very much less than those of Hb S disease and why Hb-S/Hb C disease and Hb S/thalassæmia occupy an intermediate position In Hb S disease sickling takes place at physiological degrees of deoxygenation but in the trait this is not so

The recent papers by Harris and co workers (1956) and Griggs and Harris (1956) bring together many observations on the consequences of sickling in Hb S disease and its variants and on the relationship between sickling and increases in blood viscosity and mechanical fragility A vicious circle is thought to occur in organs where the circulation is slowed and decrease in pH due to excess of carbon dioxide makes the sickling worse by facilitating deoxygenation thromboses at first plugs of erythrocytes are the result The impacted mechanically fragile cells undergo lysis and if they escape they probably circulate as irreversibly sickled cells (see also Watson (1948) and Shen Fleming and Castle (1949)) Probably too metabolic changes occur in the stagnant blood which lead to a reduction in erythrocyte life span—the small tails of osmotically fragile cells seem to represent cells which have been altered in this way

Allison s (1956b) work provides perhaps an explanation as to how Hb S disease is compatible with life in view of the fact that sickling takes place at physiological oxygen tensions He found that 2 minutes was the minimum time required for sickling *in vitro* a length of time which is fortunately much longer than the period during which the erythrocytes would be normally exposed to the oxygen tension of mixed venous blood (about 15 seconds)

Greenberg Kass and Castle (1957) showed that the clinical phenomena in Hb S disease and its variants can be correlated with the mean corpuscular concentration of Hb S (MCSHC) patients with MCSHC < 15 g per 100 ml cells were not anæmic and did not have painful crises those with a MCSHC of 15–18 g per 100 ml had a mild hæmolytic anæmia and those with a MCSHC of 20 g per 100 ml or more had significant anæmia bony lesions and painful crises Greenberg and Kass (1958) in their most recent paper consider the role of pH and other metabolic changes in the genesis of crises They believe that metabolic acidosis can induce crises—with an increase in blood viscosity and an increased number of irreversibly sickled cells in the peripheral blood presumably by facilitating sickling either locally or generally They also make the point that this may be the mechanism of the crises which sometimes follow anæsthesia (see also p 256)

support the concept of gel formation rather than of actual crystallization Perosa and co workers (1957) nevertheless have suggested that in Hb S trait the ' holly leaf type of sickling depends upon gel formation of Hb S plus actual crystallization of Hb A

Mechanism of Hæmolysis and Thrombosis *in Vivo*

It is now generally agreed that sickling *in vivo* is the cause of both the hæmolytic and thrombotic manifestations in the sickle cell syndromes Sydenstricker (1924) was perhaps the first to realize that the anæmia was hæmolytic he however considered that the changes in the erythrocytes which predisposed them to hæmolysis were secondary to a hereditary defect of the spleen and blood forming organs Hahn and Gillespie (1927) more correctly concluded that hypersplenism was unlikely they stated that the spleen merely performs its usual function in sickle cell anemia, that of capturing and destroying damaged or abnormal red blood corpuscles and that in performing this office it is damaged itself

In 1940 Ham and Castle suggested that sickling *in vivo* as the result of the deoxygenation of blood led to increases in blood viscosity which in turn caused slowing of the circulation and thus set in motion a vicious circle of further deoxygenation and increased viscosity and so on They suggested that the consequent erythrostasis would explain both the increased hæmolysis and the thromboses which occurred Shen Castle and Fleming (1944) demonstrated that sickled cells were unusually easily lysed by mechanical trauma

Bauer (1940) in a thoughtful review of the pathogenesis of the disease had come to much the same conclusion as had Ham and Castle namely that the essential process was stagnation and conglutination of disfigured red corpuscles and *that this occurred* particularly but not exclusively in those organs in which the rate of blood flow and the oxygen tension was unusually low The consequences of the stagnation he listed as (1) thrombosis *(2) ischemia necrosis and fibrosis and (3) resolution* of the red blood cells with subsequent anemia Bauer also suggested that as anæmia in his view was not the most dangerous consequence of sickling sickle cell disease was a more logical term than sickle cell anæmia in this he anticipated the report quoted on p 245 by about 17 years!

More recent work has confirmed and elaborated these early ideas As already mentioned the differences in the degree to which oxygen

readily *i e* in pure Hb S disease In Hb-S/Hb C disease and in Hb-S trait it seems to need an added factor of generalized hypoxia as in unpressurized aeroplane flights or under experimental conditions (Levin *et al* 1957) for this to happen The spleen thus acts as an involuntary storehouse for sickled cells and in doing so increases to a small extent the rate at which sickled cells are destroyed in the body But as Hahn and Gillespie pointed out in 1927 in performing this office it is damaged itself

Auto-antibodies Anti-erythrocyte auto-antibodies do not seem to play any role in the pathogenesis of the hæmolysis in Hb-S disease The work of Schneider and Levin (1950) who reported finding abnormal agglutinins in the sera of 13 patients with sickle-cell disease does not seem to have been confirmed

Pathogenesis of Hb C Disease etc Little is known as to the cause of the minor degrees of increased hæmolysis which take place in patients homozygous for Hb C Sickling does not occur this is presumably why thrombotic phenomena are absent Splenomegaly is usual and congestion of the pulp seems to be the principle cause of this (Singer Chapman *et al* 1954 Wheby Thorup and Leavell 1956)

How the congestion is brought about remains a mystery Lewis Anderson and Baskind (1957) reported the results of autohæmolysis studies on three homozygotes and one subject with the Hb-C trait In each case the addition of glucose to the blood did not diminish the hæmolysis as it normally does The significance of these observations is obscure they do however suggest the presence of some metabolic difference between cells containing Hb-C and normal cells Whether the target shape is in any way related to a defective or abnormal metabolism is unknown

More recently Smith and Krevans (1959) have suggested that the tendency to intracellular crystal formation in Hb-C disease (see p 275) may be important They point out that a higher proportion of cells containing crystals may be found in films of blood from the splenic pulp compared with peripheral blood, and that the rigidity of the rod shaped cells containing crystals may have something to do with their retention within the spleen.

TREATMENT OF SICKLE CELL DISEASE AND ALLIED DISORDERS

Although nothing as yet can be done to correct the formation of abnormal hæmoglobins palliative measures are important and deserve brief consideration

Good accounts of the early attempts at therapy are to be found in the reviews of Margolies (1951) and Leavell (1954) The use of oxygen vasodilator drugs anticoagulants steroids cobalt

McKellar and Dacie (1958) in the course of investigating the plasma thromboplastic activity in paroxysmal nocturnal hæmoglobinuria found in each of three cases of Hb S disease that incubation of whole blood *in vitro* for 1 hour at 37° C was also followed by an increase in plasma thromboplastic activity irrespective of the presence of blood platelets The thromboplastic activity was apparently derived from the erythrocytes themselves perhaps as a prehæmolytic phenomenon This observation may explain how and why thromboses occur *in vivo* in the vicinity of impacted and stagnant erythrocytes

Chemical Changes in Sickled Erythrocytes

Tosteson Shea and Darling (1952) showed that sickled cells quickly lost major amounts of potassium and gained sodium on incubation *in vitro* and in a further paper Tosteson Carlsen and Dunham (1955–56) concluded that these changes could not be accounted for by the intracellular plasma lying between centrifuged sickled cells being *increased in amount (Clarkson and Maizels 1955) Adams (1957)* nevertheless considered that the difficulty in packing sickled cells was in fact responsible for these differences These observations if substantiated suggest that the surface structure of erythrocytes may be damaged when the cells sickle they indicate an additional possible mechanism by which the life span of the erythrocytes may be shortened in sickle cell disease It may be added that although Bartlett and co workers (1955) could not demonstrate any abnormality in the carbohydrate metabolism of sickled cells Prankerd (1955) reported that studies with ^{32}P labelled orthophosphate showed that when cells sickled there was a slowing of ^{32}P uptake a fall in ATP and a diminution in the specific activity of intracellular esters

Role of the Spleen As previously indicated (p 260) in Hb S disease splenomegaly in the young subject is followed sooner or later by infarction and contraction of the spleen It is also common knowledge that splenectomy is associated at the best with a minor improvement in the patients condition The early splenomegaly is due to the splenic pulp being engorged with blood (see p 261) and there is experimental evidence that Hb S disease erythrocytes are selectively trapped in the spleen pulp (Weisman *et al* 1954) Presumably the cells become sickled there as the result of anoxia and find it difficult because of their shape to regain the general circulation It is known that the proportion of irreversibly sickled cells in the spleen pulp is raised (Watson Lichtman and Shapiro 1956 Harris *et al* 1956) presumably these are cells which have been sequestered in the pulp for a relatively long time Because the spleen pulp acts as a cul de sac it is easy to understand how infarction eventually occurs in patients whose cells sickle the most

the patients so treated were relieved of their pain *In vitro* small decreases in pH produce a marked increase in the degree of sickling and it was hoped that the reverse effect could be obtained *in vivo* if sufficient alkali was given

Blood Transfusions These are of temporary value and in severely anæmic patients they may be life saving However if the hæmoglobin is maintained at or above 7 0 g per 100 ml it would seem unwise to undertake periodic transfusion unless there is some special indication such as pregnancy

If large volumes of blood are given it is possible to depress erythropoiesis and to convert the patients blood picture temporarily almost to normal (Donegan MacIlwaine and Leavell 1954 Nadel and Spivack 1958) Under these circumstances major surgery can be attempted As already mentioned (p 255) patients with Hb-S disease or Hb-S/Hb-C disease are prone to suffer from crises as the result of anæsthesia (Shapiro and Poe 1955) Blood transfusion before operation will reduce this risk by diminishing the proportion of cells present capable of undergoing sickling Transfusions have also been reported in association with bed rest to be useful in promoting healing of chronic ulcers of the leg (Chernoff Shapleigh and Moore 1954)

Splenectomy This has been carried out on numerous occasions but the results have generally been disappointing The operation is however not entirely useless in carefully selected patients

According to Margolies (1951) splenectomy was first carried out by Hahn and Gillespie (1927) and by Stewart (1927) Shotton Crockett and Leavell (1951) reviewed the results of the operation in 24 cases The symptoms of 15 patients became less severe and there was some improvement in their erythrocyte counts of the others four patients improved slightly and four were not benefited The best results seem to have been obtained in patients who had the largest spleens *i.e* when the operation was undertaken at a relatively early stage of the disease Dickstein and Koop (quoted by Margolies (1951)) for instance carried out splenectomy in 16 children ranging from 14 months to 6 years of age and followed their progress for 1–4 years after operation Two of the children were not improved but the other fourteen did relatively well their hæmoglobins were maintained at slightly higher levels than before operation and none had a major crisis following splenectomy

Similar results have been observed in more recent series Watson, Lichtman and Shapiro (1956) referred to six patients with particularly large spleens who needed frequent transfusions. In two of them it was

acetazolamide alkalies blood transfusions and splenectomy will be considered briefly in the following sections The remarks are mainly concerned with Hb S disease itself in Hb S/Hb C disease etc the problem is less serious

Oxygen therapy seems to be contraindicated Reinhard Moore Dubach and Wade (1943) concluded that prolonged administration of oxygen did not inhibit hæmolysis and did not relieve pain Moreover it inhibited compensatory erythropoiesis to some extent with the result that the patients became more anæmic

Vasodilator drugs such as Priscoline have been used in the treatment of abdominal pain on the hypothesis that the pain is due to vascular spasm Smith Rosenblatt and Bedo (1953) reported good results in seven children Further information is required

Anticoagulants do not seem to have been extensively tried Griffiths (1955) however reported an instance in which Tromexan therapy appeared to be useful It seems however unlikely that anticoagulants could achieve more than minor improvement at the best as their administration probably does not affect the process of sickling itself

Steroid hormones do not seem likely to be of any value in treatment Kass and co workers (1951) treated two patients with ACTH Crises followed on two occasions in the first patient and in the second patient a mild crisis was precipitated which was followed by remission and a rise in the erythrocyte count Sass (1952) however reported dramatic symptomatic improvement in one patient but without any significant change in the blood count

Cobalt seems to be both ineffective and dangerous Wolf and Levy (1954) treated four patients with 300 mg daily although the results were considered encouraging gastro intestinal irritation followed and the treatment had to be stopped Gross Kriss and Spaet (1955) reported the results of treating four children temporary rises in hæmoglobin were followed by relapse in three of them and all three developed goitres one having symptoms of severe myxœdema

Acetazolamide (Diamox) a carbonic anhydrase inhibitor has been reported by Hilkovitz (1957) to inhibit sickling both *in vitro* and *in vivo* Whether however this will prove to be a practicable and worth while method of treatment in doses that can be tolerated by the patient remains to be demonstrated Gailitis and co workers (1957) reported no benefit in one case

Alkalies given intravenously have been used by Greenberg and Kass (1956 1958) in the treatment of crises Some but not all of

AKSOY M (1955) Sickle cell trait in South Turkey *Lancet* **1** 589
AKSOY M (1956) Sickle cell anæmia in South Turkey a study of fifteen cases in twelve white families *Blood* **11** 460
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ASCENZI A and SILVESTRONI E (1953) On the optical properties of the hemoglobin in microdrepanocytic disease *Blood* **8** 1061
ASCENZI A and SILVESTRONI E (1957) Étude anatomoclinique d un cas de maladie microdrepanocytique *Acta hæmat (Basel)* **18** ?05
BANKS L O SCOTT R B and SIMMONS J (1952) Studies in sickle cell anemia Inheritance factor including effect of interaction of genes for sicklemia and thalassemia *Amer J Dis Child* **84** 601

possible to demonstrate that the survival of transfused normal erythrocytes was impaired which suggested that an extravascular factor contributed to the hæmolysis Splenectomy in these two patients was of striking clinical benefit and this was associated with a return to normal of the survival of transfused normal cells

As already referred to in discussing erythrocyte life span Sprague and Paterson (1958) reported that the ^{51}Cr half times of tagged erythrocytes more than doubled as a result of splenectomy This was associated with a rise in the mean hæmoglobins of the patients from 5 6 g per 100 ml to 8 3 g per 100 ml and a reduction in the number of hospital admissions from 26 per 19 5 patient years to 2 per 12 5 patient years A further seven patients were mentioned who had *already been splenectomized and who after operation no longer needed* transfusions

The reports mentioned above show not only that splenectomy can be carried out successfully in young children with Hb S disease but that considerable clinical and hæmatological benefit may be expected if the spleen is large Clearly if a child has to be maintained on transfusions and his spleen is palpable splenectomy should be seriously considered In older children and adolescents when the spleen has become fibrosed and is no longer palpable there would seem to be no case for contemplating splenectomy

Splenectomy has also been carried out in patients with Hb S/Hb C disease Hb S/thalassæmia and in Hb C disease (Singer Chapman *et al* 1954 Wheby Thorup and Leavell 1956) Less is known about the results of the operation than in Hb S disease It seems probable however that the benefit if any is slight

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